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Metabolism Clinical and Experimental 60 (2011) 92 – 98
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Metabolic response to endotoxin in vivo in the conscious mouse: role of interleukin-6

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Received 19 June 2009; accepted 21 December 2009

Abstract

Inflammation and insulin resistance are characteristics of endotoxemia. Although the role of interleukin (IL)-6 in insulin-resistant states has been characterized, little is known of its role in the metabolic response to inflammation. To study the role of IL-6, conscious chronically catheterized mice were used. Five days before being studied, catheters were implanted in the carotid artery and jugular vein. After a 5-hour fast, *Escherichia coli* (250 μg per mouse) lipopolysaccharide (LPS) was injected in IL-6$^{-/-}$ (KO, n = 13) and IL-6$^{+/+}$ (WT, n = 10) littermates. The IL-6 response to LPS was simulated in an additional group of KO mice (KO + IL-6, n = 10). Interleukin-6 increased in WT (15 ± 0.7 ng/mL) 4 hours after LPS and was undetectable in KO. Interleukin-6 replacement in the KO restored circulating IL-6 to levels observed in the WT group (14 ± 0.3 ng/mL). Tumor necrosis factor–α increased more rapidly in WT than in both KO and KO + IL-6 mice. The KO mice exhibited a more profound glucose excursion 30 minutes after LPS injection and no apparent hypoglycemia at 4 hours (95 ± 5 vs 70 ± 8 mg/dL, KO vs WT), despite having a blunted glucagon and epinephrine response. Glucose levels in KO + IL-6 mice, while decreased (93 ± 4 mg/dL) at 4 hours, remained higher than those in WT mice. In summary, the absence of IL-6 protected against LPS-induced hypoglycemia. Acute restoration of the IL-6 response to LPS did not potentiate hypoglycemia but partially restored the glucagon response. Thus, although IL-6 promotes glucose intolerance in insulin-resistant states, IL-6 promotes hypoglycemia during acute inflammation.

1. Introduction

The pathogenic effects of gram-negative bacteria are primarily brought about by exposure within the mammalian host to the bacterial endotoxic component called *lipopolysaccharide* (LPS). Exposure to LPS results in a subsequent chain of events that varies widely but can consist of infection, septic shock, and eventually death [1]. Patients with septicemia experience an increase in the mobilization and utilization of glucose in conjunction with a major influx of immunologic factors. One important feature of the hypermetabolic response in vivo is an increase in the synthesis and release of glucose from the liver early in response to an LPS challenge [2,3]. This generally leads to a brief state of hyperglycemia coupled with elevated insulin levels followed by, in some cases, hypoglycemia. The severity of the hypoglycemia is dependent upon the dose of LPS used, the species, and the strain [2,4]. Hypoglycemia develops despite marked insulin resistance, increased gluconeogenic precursor supply (lactate and gluconeogenic amino acids), and increased levels of counterregulatory hormones [3].

Lipopolysaccharide activates the release of many pro- and anti-inflammatory cytokines. Tumor necrosis factor (TNF)–α is typically the first cytokine to appear in the circulation after LPS challenge and is thought to play a major role in lethality after endotoxic shock [5]. This is followed shortly by other proinflammatory and anti-inflammatory cytokines including interleukin (IL)-1β, IL-6, IL-10, IL-12, and interferon-γ [6].

Glucose uptake is increased in many tissues especially those rich in macrophages after an LPS challenge. Interestingly, in rats in vivo, LPS has been shown to increase whole-body glucose disposal. The enhanced glucose uptake

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0026-0495/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.12.022

by the periphery is distributed to barrier tissues such as the skin and lung as well as the spleen [7-10]. Tumor necrosis factor stimulates the same effect, leading one to speculate that this cytokine may play a crucial role in alterations in glucose uptake; yet neutralization of TNF does not completely block the overall metabolic response to endotoxin challenge [11] Thus, it is likely that cytokine release after endotoxin exposure may be one of the mechanisms by which LPS exerts its effects on glucose metabolism [10]. Although many studies have been done in a number of species, the impact of LPS on tissue glucose uptake in the mouse has not been characterized.

It has been suggested that IL-1 contributes to the hypoglycemia seen during endotoxemia and IL-6 plays a lesser role [12]. However, IL-6 is a unique cytokine exhibiting both pro- and anti-inflammatory properties depending on the target cell type. Physiologic stimuli for the synthesis of IL-6 include IL-1, TNF, and bacterial endotoxin, with levels increasing up to 300-fold during endotoxic insult. The lack of marked effects of IL-6 deficiency on glucose concentration may in part be due to offsetting effects of IL-6 on glucose kinetics. Interleukin-6 can both augment and impair insulin action in vivo [13-18].

The pro- and anti-inflammatory nature of IL-6 adds many layers of complexity to discerning its role in a pathogenic state such as sepsis. There is a profound lack of data correlating the interaction of increased levels of IL-6 during inflammation with alterations in carbohydrate flux. This study examined the impact of IL-6 on the metabolic response to LPS in vivo in the presence and absence of the IL-6 protein.

2. Methods

2.1. Animal care and husbandry

All procedures were performed on IL-$6^{-/-}$ mice on a C57BL/6 background (B6.129S2-*Il6*^{tm1Kopf}/J; Jackson Laboratory, Bar Harbor, ME) that were backcrossed to C57BL/6J mice for 5 generations to obtain 2 genotypes: $IL6^{-/-}$ and $IL6^{+/+}$. Genotyping of mice was performed as described on the Jackson Laboratory Web site. At 3 weeks of age, mice were separated by sex and maintained in microisolator cages on a 12-hour light/dark cycle with free access to food and water. All experiments were performed at approximately 4 months of age. All procedures performed were approved by the Vanderbilt University Animal Care and Use Committee.

2.2. Surgical procedures

The surgical procedures used to implant chronic catheters have previously been described [19,20]. Briefly, mice were anesthetized with an injection of pentobarbital (70 mg/kg body weight). The left common carotid artery and right jugular vein were catheterized for sampling and infusions, respectively. The free ends of the catheters were tunneled under the skin to the back of the neck where the loose ends of the catheters were attached via stainless steel connectors to tubing made of Micro-Renathane (0.033-in outer diameter); Braintree Scientific, Braintree MA), which was exteriorized and sealed with stainless steel plugs. Animals were individually housed after surgery, and body weight was recorded daily. The lines were flushed daily with approximately 50 μL of saline containing 200U/mL of heparin and 5 mg/mL of ampicillin.

2.3. In vivo metabolic experiments

All metabolic experiments were performed after an approximately 5-day postoperative recovery period, depending on restoration to 10% of presurgery body weight. For metabolic studies, conscious, unrestrained mice were placed in approximately 1-L plastic container lined with bedding and fasted for 5 hours. Approximately 2 hours before the experiment, the animals were connected to a dual-channel stainless steel swivel (Instech Laboratories, Plymouth Meeting, PA.) to allow simultaneous jugular vein infusion and sampling of arterial blood. A 5-μCi bolus of 3-[^{3}H]-D-glucose was then given into the jugular vein followed by a constant infusion at a rate of 0.05 μCi/min. Mice were not handled and allowed to move freely to eliminate stress. Approximately 1 hour before an experiment, red blood cells from a donor mouse on a C57Bl/6 background washed with 0.9% heparinized saline were infused at a rate of 2 μL/min for the duration of the study to minimize falls in the hematocrit. After a 2-hour equilibrium period (t = 0), a baseline arterial blood sample was drawn for measurement of blood glucose, 3-[^{3}H]-D-glucose, hematocrit, plasma insulin, plasma glucagon, catecholamines, and cytokines.

The response to an LPS challenge was examined in 3 groups of mice: $IL6^{-/-}$ (KO, n = 13), $IL6^{+/+}$ (WT, n = 10), and $IL6^{-/-}$ with replacement of IL-6 to stimulate the natural IL-6 response observed in WT (KO + IL-6, n = 10). At t = 5 minutes, mice were given a 250–μg per mouse bolus of *Escherichia coli* endotoxin (LPS), followed by 25 μL saline (or saline alone) that was given into the jugular vein catheter. In KO + IL-6 mice, immediately after the bolus of endotoxin, a constant infusion of rmIL-6 (16 μg/h) (Research Diagnostics, Flanders, NJ) at a rate of 1 μL/min was begun; and the clock was reset.

At t = 30 minutes, the infusion of rmIL-6 was increased to 32 μg/h (pump rate = 2 μL/min) for the remainder of the study. In all groups at t = 30 minutes, arterial blood was sampled to determine blood glucose, 3-[^{3}H]-D-glucose, hematocrit, plasma insulin, glucagon, epinephrine, norepinephrine, and cytokines. Arterial blood samples were taken at t = 60,120,180, 210, and 240 minutes to determine blood glucose, 3-[^{3}H]-D-glucose, and cytokine levels. At t = 240 minutes, a 13-μCi bolus of 2-deoxy [^{14}C] glucose ([2-^{14}C] DG) was administered into the jugular vein catheter. At t = 242, 245, 250, 255, and 265 minutes, arterial blood was sampled to determine blood glucose and plasma 3-[^{11}]-D-

glucose and [2-^{14}C]DG. At t = 265 minutes, a final arterial blood sample was taken to assess all hormones and plasma [2-^{14}C]DG. The mice were then anesthetized. The soleus, gastrocnemius, superficial vastus lateralis (SVL), omental adipose tissue, liver, kidney, duodenum, jejunum, diaphragm, heart, subcutaneous adipose tissue, and brain were excised; immediately frozen in liquid nitrogen; and stored at −70°C until future tissue analysis.

2.4. Plasma and muscle sample analysis

Immunoreactive insulin and glucagon were assayed using a Linco Rat Radioimmunoassay kit (Linco Research, St Charles, MO) and a double-antibody method. Plasma epinephrine and norepinephrine levels were assessed by high-performance liquid chromatography with electrochemical detection after alumina extraction, elution, and preconcentration. Cytokines were analyzed using multiplexing Luminex xMAP technology (Luminex, Austin, TX). Plasma samples were prepared using mouse cytokine/chemokine Lincoplex kit (Linco Research). The plasma samples were deproteinized, and both [2-^{14}C]DG and [2-^{14}C]DG-G-phosphate ([2-^{14}C]DGP) radioactivity levels of the excised tissues were determined using liquid scintillation counting (Beckman LS 3801; Beckman Instruments, Irvin, CA).

2.5. Calculations

Glucose flux rates were assessed using non–steady-state equations [21] assuming a volume of distribution (130 ml/kg). Tissue-specific clearance (K_g) of [2-^{14}C]DG and glucose uptake (R_g) were calculated as previously described [22]:

$$K_g = \left[2\text{-}^{14}\text{C}\right]\text{DGP}_{\text{tissue}} / \text{AUC}\left[2\text{-}^{14}\text{C}\right]\text{DG}_{\text{plasma}}$$

$$R_g = \left(K_g\right)\left(\left[\text{glucose}\right]_{\text{plasma}}\right)$$

where [2-^{14}C]DGP$_{tissue}$ is the [2-^{14}C]DGP radioactivity (in disintegrations per minute per gram) in the tissue, AUC [2-^{14}C]DG$_{plasma}$ is the area under the plasma [2-^{14}C]DG disappearance curve (in disintegrations per minute per milliliter per minute), and [glucose]$_{plasma}$ is the average blood glucose (in micrograms per microliter) during the experimental period. All data are presented as mean ± SEM, and the significance level was set at $P < .05$.

3. Results

3.1. Cytokine concentration

Blood IL-6 levels rose rapidly in WT after LPS injection. As expected, IL-6 was undetectable in KO mice. The IL-6 profile in the KO + IL-6 mice was successfully matched to the WT mice (Fig. 1).

The TNF-α levels were low in the basal period for all 3 groups. After LPS injection, the TNF-α levels increased to approximately 1000 pg/mL by 30 minutes in WT mice and remained elevated for the remainder of the study. The rate of rise of TNF-α was slower in KO and KO + IL-6 mice, not reaching comparable levels until 180 minutes. Interestingly, in KO mice, TNF-α levels rapidly declined after 180 minutes (Fig. 1). Interleukin-10 increased to equivalent levels in all 3 groups by 180 minutes after LPS injection, although IL-10 rose earlier in KO. Interleukin-1β increased in all groups to a similar extent (Fig. 2). Saline injection did not alter any of the cytokines in WT (data not presented).

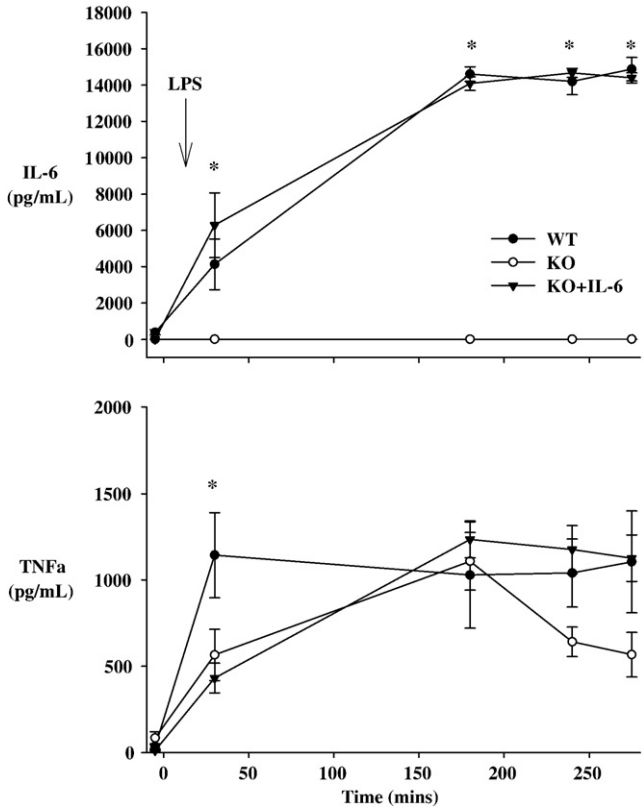

Fig. 1. The plasma concentration of IL-6 and TNF-α (in picograms per milliliter) in WT, KO, and KO + IL-6 mice. Data are expressed as mean ± SEM. $P < .05$ WT vs KO.

3.2. Glucose metabolism

Arterial blood glucose concentrations were similar during the basal period for KO and KO + IL-6, whereas WT mice had a significantly lower basal arterial glucose concentration (Fig. 3). Within the first 60 minutes after LPS injection, the arterial glucose concentration peaked for all 3 groups. Subsequently, glucose levels began to decrease, falling below baseline for all groups by 240 minutes, with the WT mice reaching glucose concentrations deemed hypoglycemic (70 ± 8 mg/dL). Associated with this gradual fall in glucose was a progressive rise in glucose clearance above the basal rate in all 3 groups. The rate of glucose production (R_a) and glucose disappearance (R_d) remained similar between the 3 groups throughout the study (Fig. 4). In all 3 groups, the administration of LPS inhibited tissue

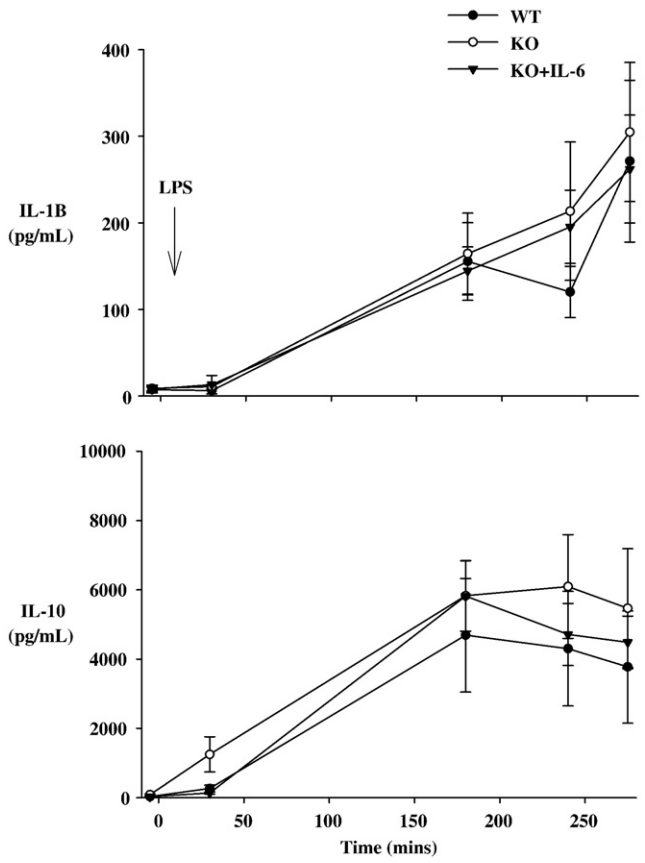

Fig. 2. The plasma concentration of IL-1β and IL-10 in LPS-injected WT and KO mice, and KO receiving LPS and a simulated IL-6 infusion. Data are expressed as mean ± SEM.

glucose uptake (R_g) in the diaphragm, heart, and intrascapular adipose tissue but not in other tissues studied compared with control animals. Replacement of IL-6 in the KO mice led to decreased R_g in the liver (Fig. 5). These results were recapitulated in the tissue-specific glucose clearance (K_g, data not shown).

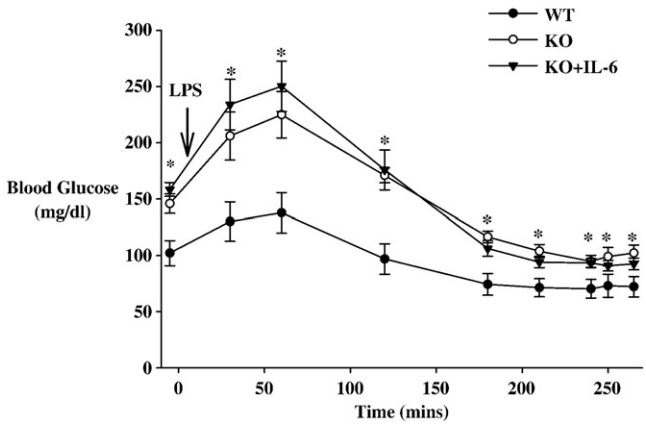

Fig. 3. Arterial blood glucose concentration in LPS-injected WT and KO mice, and KO receiving LPS and a simulated IL-6 infusion. Data are expressed as mean ± SEM. $*P < .05$ vs WT.

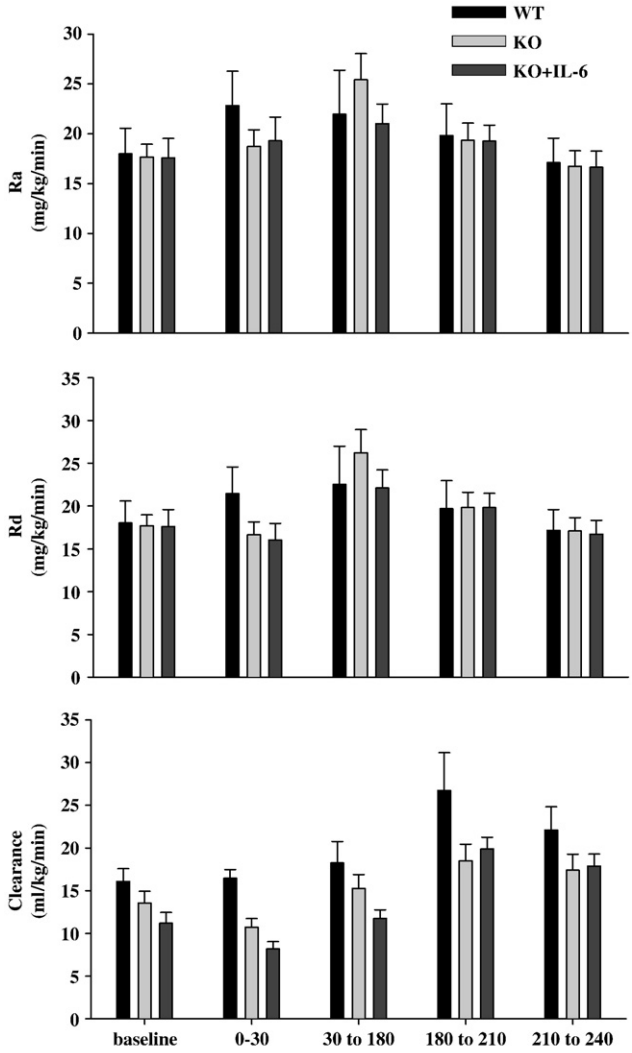

Fig. 4. The rates of whole-body glucose appearance (R_a) and disappearance (R_d) in LPS-injected WT and KO mice, and KO receiving LPS and a simulated IL-6 infusion. Data are expressed as mean ± SEM.

3.3. Hormone concentrations

During the basal period, insulin levels were the same in the WT and KO + IL-6 replacement groups (Table 1). However, insulin levels in the KO mice were significantly higher. After LPS, insulin levels gradually decreased in all groups for the remainder of the study. Glucagon and epinephrine levels increased in WT after LPS, whereas they failed to increase in KO. Infusion of IL-6 in KO partially restored the glucagon but not the epinephrine response to LPS injection.

4. Discussion

Using our chronically catheterized conscious mouse model, we assessed the endocrine, metabolic, and cytokine response to LPS and the impact of the IL-6 deficiency. As

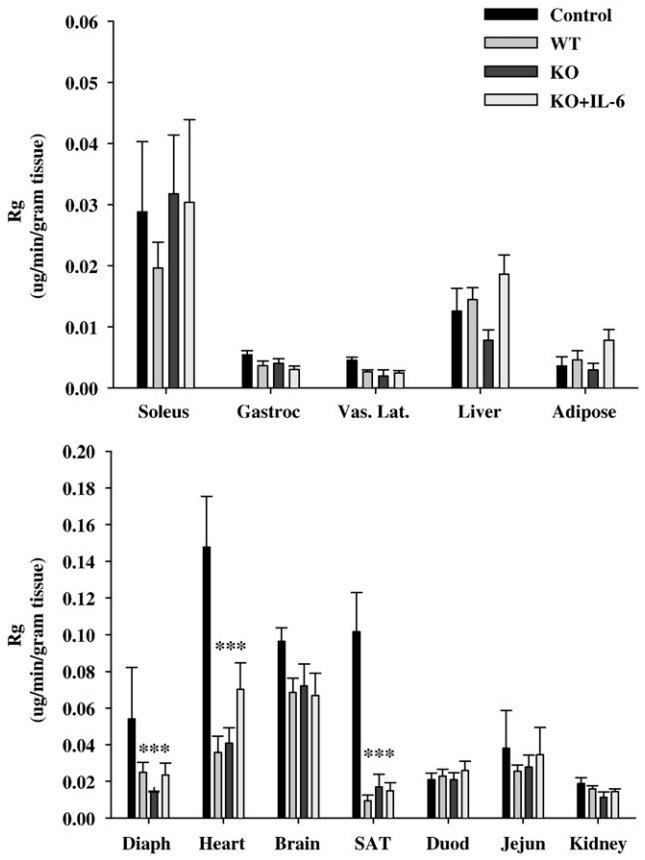

Fig. 5. The rates of tissue (soleus, gastrocnemius, white vastus lateralis, liver, gonadal adipose tissue, diaphragm, heart, brain, intrascapular adipose tissue, duodenum, jejunum, and kidney) glucose uptake (R_g) in saline-treated wild-type (control) animals and LPS-injected WT and KO mice, and KO receiving LPS and a simulated IL-6 infusion. Data are expressed as mean ± SEM. $*P < .05$ vs control.

shown in multiple species, LPS administrations lead to a transient increase in glucose concentration [8]. In our wild-type mice, this increase in the arterial glucose concentration was followed by a gradual fall to induce mild hypoglycemia. In IL-$6^{-/-}$ (KO) mice, basal glucose concentration was increased, which limited the extent of LPS-induced hypoglycemia. Interestingly, this increase occurred despite an absent epinephrine and glucagon response to LPS in KO. The acute replacement of IL-6 to restore the IL-6 response to endotoxin in KO mice did not alter the metabolic response to LPS. The transient rise in glucose concentration was primarily due to a rise in glucose appearance likely reflecting a rise in hepatic glucose production. Lipopolysaccharide did not increase and in fact decreased glucose uptake in a number of tissues, which was not altered by the absence of IL-6. Despite attenuating the neuroendocrine response to LPS, the absence of IL-6 does not markedly alter the metabolic response.

As glucose production waned over time, whole-body glucose clearance gradually rose, which explained the gradual development of mild hypoglycemia. In humans, endotoxemia results in a hypermetabolic response characterized by an increase in glucose synthesis and release from the liver, leading to a brief state of hyperglycemia coupled with an elevated insulin concentration [23,24]. This has been shown to lead to a state of insulin resistance [7]. In rats, Lang et al [2] demonstrated that the changes in glucose kinetics are dependent on the dose of LPS. The extent of the early rise in glucose increased as the dose of LPS was increased. However, the severity of the hypoglycemia also was exacerbated at high doses of LPS. In dogs, the hyperglycemic phase is less robust; and hypoglycemia develops more readily [3]. Our studies reveal a slightly different mechanism in the mouse. Although all groups experienced an early transient hyperglycemia, we did not see an early elevation in insulin as has been reported in rats. The explanation for the differing kinetics in mice is unclear. The studies were done in a glycogen-replete state, and the likely mechanism for the rise in glucose production is a rise in hepatic glycogenolysis [3]. Not surprisingly, both epinephrine and glucagon increased, which likely contributed to the initial rise in glucose flux [25].

Table 1
Plasma insulin, glucagon, and catecholamine response to LPS in chronically catheterized conscious WT, KO, and KO + IL-6

Time (min)	0	30	265
Insulin (ng/mL)			
WT	0.5 ± 0.1	0.4 ± 0.1	0.2 ± 0.1
KO	0.7 ± 0.1*	0.7 ± 0.1	0.4 ± 0.1
KO + IL-6	0.5 ± 0.1	0.5 ± 0.3	0.3 ± 0.1
Glucagon (pg/mL)			
WT	157 ± 31	212 ± 56*	243 ± 60
KO	53 ± 10	98 ± 11	97 ± 13
KO + IL-6	71 ± 18	127 ± 30	285 ± 40
Epinephrine (pg/mL)			
WT	70 ± 26	243 ± 75	522 ± 145*
KO	22 ± 2	137 ± 39	109 ± 40
KO + IL-6	41 ± 10	89 ± 27	63 ± 18
Norepinephrine (pg/mL)			
WT	124 ± 27	244 ± 22	372 ± 60
KO	137 ± 19	224 ± 57	206 ± 42
KO + IL-6	226 ± 25	153 ± 15	208 ± 24

Data are expressed as mean ± SEM.
* $P < .05$

The basal glucose concentration and the glucose excursion after LPS injection were exaggerated in IL-6 KO. This observation was interesting, as studies conducted by Di Gregorio et al [26] have concluded that both WT and KO mice after a 4-hour fast had similar glucose levels, which ranged just below 200 mg/dL. The absence of IL-6 resulted in a more robust early hyperglycemic response to LPS and the absence of subsequent hypoglycemia. One might expect that the absence of IL-6 would improve insulin action, as acute infusion of IL-6 impairs insulin action [14,27]. Although basal glucose concentrations were elevated in KO, the insulin concentration was not increased or only modestly increased, suggesting only mild insulin resistance. However, given the higher glucose concentration, it is possible that insulin resistance combined with altered

pancreatic endocrine function may contribute to the differing glucose concentration.

The absence of IL-6 delayed the TNF-α and hastened the IL-10 response to LPS. Whereas the KO mice have no IL-6 paracrine or endocrine activity, the WT mice experienced an increase in both the paracrine and endocrine activity during exposure to LPS, which is in part mediated through the up-regulation of TNF-α observed during the early infection phase. As expected, our WT mice had a significant increase ($P < .03$) in TNF-α within the first 30 minutes after the LPS bolus, which was accompanied by a characteristic rise in IL-6. Fattori et al [28] reported that, in the absence of IL-6, there was a 3-fold increase in TNF-α in these animals. Although our KO mice experienced an increase in TNF-α as well, this increase was delayed as compared with the WT mice. Because IL-6 can amplify TNF-α transcription, the absence of IL-6 in the KO mice may explain the observed delay in the TNF-α response [5].

The anti-inflammatory cytokine IL-10 has been associated with the counterregulatory effects of both IL-6 and TNF-α [29] and works primarily to quell the possible detrimental effects of these cytokines. Our finding draws a parallel with previous studies in that, during early endotoxemia, IL-10 activation had a sluggish response during the time when TNF-α and subsequently IL-6 were first activated in WT mice. There is speculation that the early increase in the proinflammatory cytokines may actually suppress the beneficial early activation of IL-10 [29]. Interestingly, our KO mice experienced an early activation of IL-10 and a delayed activation of TNF-α, which we believe are facilitated by the absence of the rise of IL-6, as IL-6 replacement delayed the IL-10 response. The beneficial effects of IL-10 may be masked by the increased levels of IL-6 observed during endotoxemia. These finding correlate with previous studies conducted where anti–TNF-α antibodies were given before a bolus of LPS; IL-6 and IL-1β were blunted, whereas IL-10 had an increased early response [30].

We infused rmIL-6 into KO mice in an attempt to restore the endocrine effects observed in WT mice to LPS. Although we successfully recreated the IL-6 profile observed in the WT mice, the infusion of IL-6 did not correct the delayed TNF-α or IL-1β response to LPS; but it did delay the IL-10 response. This could be explained by the need of the endocrine levels of IL-6 to reach a certain level to trigger the positive feedback response previously mentioned. This is especially relevant, as the TNF-α levels in the KO + IL-6 group closely mirrored the levels observed in WT mice from 180 minutes until the end of the study. These mice also displayed the same sluggish IL-10 response, suggesting that the endocrine effects of IL-6 are an important modulator of the IL-10 response.

As previously mentioned, KO and KO + IL-6 mice had similar fasting basal glucose concentrations and experienced an early transient hyperglycemia. We postulated that the infusion of IL-6 into the KO animals would recapitulate the immunologic and hormonal responses observed in WT animals. These mice displayed a glucose profile similar to KO throughout the entirety of the study, although there was a delayed response in both TNF-α and IL-10. This is interesting because it suggests that the endocrine actions of IL-6 are not enough to alter glucose metabolism. It is also interesting to note that both KO and KO + IL-6 had a profound decrease in glucose clearance in the first 30 minutes, whereas KO + IL-6 had a profound increase in clearance from 180 minutes until the end of the study ($P < .050$). The early decrease in clearance amplified the early rise in glucose in response to LPS in both groups.

The absence of IL-6 modulates the glucagon and epinephrine response to LPS. The KO mice experienced decreased basal glucagon concentrations and a blunted increase in glucagon and epinephrine in response to LPS. Interestingly, simulation of the IL-6 response to LPS partially restored the glucagon but not the epinephrine response to LPS, suggesting that IL-6 stimulation regulates glucagon secretion. This observation is consistent with recent work suggesting that IL-6 modulates α cell hyperplasia in response to high-fat feeding [31]. Despite IL-6 replacement partially restoring the glucagon response to LPS injection, glucose flux was not altered. This suggests that glucagon may play a minor role in sustaining glucose production after LPS.

In conclusion, the absence of IL-6 appeared to be protective against endotoxin-induced hypoglycemia. It is possible that early alterations, glycemia variables, and cytokines allowed for the late-term protective effects against hypoglycemia. Our data suggest that end-stage glucose levels observed in KO mice may be attributed to factors other than hormonal catabolic mediators such as glucagon, epinephrine, and norepinephrine, as the epinephrine and glucagon response was markedly attenuated in the KO mice. This observation, coupled with the previously stated glucose kinetic profile, leads us to believe that carbohydrate metabolism is not altered directly through the properties of the IL-6 protein. Our studies indicate that IL-6 is partly, but not entirely, responsible for the metabolic and immunologic profile seen during high-dose endotoxemia. Likewise, the WT metabolic and immunologic response could not be recreated in the IL-6 replacement group, which leads us to believe that the changes observed during endotoxemia occur indirectly either through an IL-6–mediated transcription pathway or through a redundant immunologic signaling pathway that includes IL-6.

Acknowledgment

This work was supported by National Institutes of Health Grants U24 DK-59637 and R01 DK-43748 and P30 DK-058404. Special thanks to the members of the Mouse Metabolic Phenotyping Center (Carlo Malabanan and Deanna Bracy) who implanted the catheters. Kimberly Mulligan was supported by the Molecular Endocrinology Training Grant T32-DK-07563.

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