NF-κB Dependent Airway Inflammation Triggers Systemic Insulin Resistance

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Abstract

Inflammatory lung diseases (e.g. pneumonia and acute respiratory distress syndrome) are associated with hyperglycemia, even in patients without a prior diagnosis of type 2 diabetes. It is unknown whether the lung inflammation itself or the accompanying comorbidities contribute to the increased risk of hyperglycemia and insulin resistance.

To investigate whether inflammatory signaling by airway epithelial cells can induce systemic insulin resistance, we utilized a line of doxycycline-inducible transgenic mice that express a constitutive activator of the nuclear factor kappa-B (NF-κB) in airway epithelial cells. Airway inflammation with accompanying neutrophilic infiltration was induced with doxycycline over 5 days. Then, hyperinsulinemic-euglycemic clamps were performed in chronically catheterized, conscious mice to assess insulin action. Lung inflammation decreased the whole body glucose requirements and was associated with secondary activation of inflammation in multiple tissues. Metabolic changes occurred in the absence of hypoxemia. Lung inflammation markedly attenuated insulin-induced suppression of hepatic glucose production and moderately impaired insulin action in peripheral tissues. The hepatic Akt signaling pathway was intact, while hepatic markers of inflammation and plasma lactate were increased. As insulin signaling was intact, the inability of insulin to suppress glucose production in the liver could have been driven by the increase in lactate, which is a substrate for gluconeogenesis, or due to inflammation driven signal that is independent of Akt. Thus, localized airway inflammation that is observed during inflammatory lung diseases can contribute to systemic inflammation and insulin resistance.
Introduction

Lung diseases such as asthma, chronic obstructive pulmonary disease (COPD), pneumonia, and acute respiratory distress syndrome (ARDS) feature inflammation of the airway epithelium and are associated with hyperglycemia. This hyperglycemia increases the risk of poor outcomes in hospitalized patients with lung injury (3, 6, 9, 29, 42). Hyperglycemia and insulin resistance are present almost immediately after lung injury, and before the diagnosis of ventilator-associated pneumonia (34). Even several years after an infection with pneumonia, there is an association between the disease, hyperglycemia, and increased risk of death (22). The link between hyperglycemia and pulmonary disease is poorly understood, but it is thought to be due to insulin resistance rather than beta cell dysfunction (12). The severity of inflammation during lung disease is correlated with accompanying impairments in glucose homeostasis. However, it is unclear if a primary inflammatory event localized in the airway epithelium as opposed to accompanying comorbidities (e.g. obesity, hypoxia, steroid treatment) or the presence of a specific pathogen (e.g. bacteria or virus) can impair insulin action.

Lung diseases and diabetes are both inflammatory disorders. Lung inflammation is associated with systemic inflammation (41). However lung inflammation also is accompanied with comorbidities (e.g. obesity) that also induce inflammation. It is unknown whether isolated lung inflammation in the absence of other comorbidities that are known to increase inflammation and diabetes risk can induce system inflammation and insulin resistance. We predicted that targeted airway inflammation would induce
systemic insulin resistance. We used doxycycline to induce the airway epithelium expression of a constitutively active Iκβ kinase (IKK2), an activator of the key inflammatory regulator: nuclear factor kappa-B (NF-κB), in a transgenic mouse model. When activated, IKK2 triggers the activation and nuclear translocation of NF-κB. NF-κB activates the transcription of genes involved in inflammation, such as various cytokines and chemokines. The induction of NF-κB resulted in neutrophilic and macrophage infiltrate in the lung (10). We demonstrated that acute airway inflammation, even in the absence of hypoxemia or other commodities that are commonly seen with lung disease and increases diabetes risk, induced systemic insulin resistance and inflammation.

Methods

Animal Care and Husbandry

Male mice on a C57BL/6J (Figures 2-7) or BALB/c (Figure 1) background that express an inducible constitutively active Iκβ kinase (IKK2) in the airway epithelium were used (10). The mice that expressed constitutively active IKK2 were generated after mating two different transgenic mouse lines. The first group of transgenic mice expressed a constitutively active IKK2 under the control of a tet-O promoter (cIKK2) and a gene for tTS that is driven by a CC10 promoter, which was specifically expressed in Clara cells of the lung epithelia (31). The double transgenic mouse line, was crossed with an additional transgenic mouse line that expresses a gene for rtTA that is under the control of the CC10 promoter. This cross generated a triple transgenic mouse line that expressed constitutively active IKK2 when exposed to doxycycline (denoted IKTA). Inclusion of doxycycline (1.0g/L) in the drinking water for the control mice and the IKTA
mice induced the expression of the constitutive IKK2 only in lung epithelial cells of the
IKTA mice. Splenda® (2.0g/L) was also added to drinking water. Control littermates
carried only the CC10-rtTA component. The mice were maintained in micro-isolator
cages on a 12-h light/dark cycle with free access to food and water. All experiments
were performed on mice that were approximately 3 months of age. The Vanderbilt
University Institutional Animal Care and Use Committee approved all procedures
performed.

**Surgical Procedures**

The surgical procedures for implanting chronic catheters have been described previously
(1, 2, 37). Six days before a metabolic study, mice were anesthetized with isoflurane.
The left common carotid artery and right jugular vein were catheterized allowing for
sampling and infusing, respectively. The free ends of the catheters were tunneled
subcutaneously to the back of the neck and attached via stainless steel connectors to
Micro-Renathane (0.033 in OD) tubing. The tubing was exteriorized and then plugged.
After surgery, the animals were individually housed, and body weight was allowed to
recover to within 10% of presurgical weight.

**In Vivo Metabolic Experiments**

Five days prior to metabolic experiments, chronically catheterized IKTA (C57BL/6J)
mice and controls were given access to water that contained (1.0g/L) doxycycline
(Dox). IKK2 transgene induction specifically within airway epithelium has been
previously shown to induce NF-κB activation and lung inflammation in IKTA but not
control mice. The impact on systemic inflammation was not assessed (10). After a 5 h
fast, a hyperinsulinemic-euglycemic clamp (goal glucose: 130 mg/dl) was performed to
assess insulin action in control (n=9) and IKTA (n=11) mice. A bolus of 1.0 µCi [3-³H]-
D-glucose was given (t=-120 min) followed by a constant 0.05 µCi/min infusion for 120
min until the initiation of the clamp. Clamp onset was defined as initiation of an infusion
of insulin (4 mU·kg⁻¹·min⁻¹), of glucose containing [3-³H]-D-glucose, and of
reconstituted red blood cells from a donor mouse (t=0 min). Arterial blood samples
were taken every ten minutes to determine blood glucose levels, and the glucose
infusion rate was adjusted to maintain euglycemia. After a 120 min clamp period, a 5.0
µCi bolus of [¹⁴C-2]-Deoxyglucose ([2-¹⁴C] DG) was given into the jugular vein. Blood
samples for tracer analysis were collected at t=-10, 0, 80, 90, 100, 120, 122, 125, 130,
135, and 145 min. At the end of the clamp, the mice were terminally anesthetized with
sodium phenobarbital. The soleus, gastrocnemius, superficial vastus lateralis (SVL),
liver, heart, and brain were excised, immediately frozen in liquid nitrogen, and stored at
-80°C until analysis. Plasma glucose levels, plasma glucose specific activity ([3-³H]-D-
glucose and [¹⁴C-2]-Deoxyglucose) and tissue accumulation of phosphorylated [2-¹⁴C]
DG were assessed. Tracer-determined glucose flux and tissue glucose uptake (Rg)
were calculated as previously described (1, 35).

H&E staining, BAL cell collection and counting

Mice were euthanized after 5 days of Dox. At the time of sacrifice, lungs were lavaged
(1.0 mL sterile normal saline), and then perfused and fixed in formalin for 24 hours.
After fixation, lungs were embedded in paraffin, sectioned (5 µm) and stained with
hematoxylin and eosin (H&E). The bronchoalveolar lavage (BAL) fluid was centrifuged
at 300xg for 10 min to separate cells from supernatant. Total and differential cell counts were assessed as previously described (45). Cytokine (TNF-α, IL-1β, IL-5 and IL-6) content in BAL samples and plasma was assessed using multiplex technology (MAGPIX®, Millipore Billerica, Mass).

**Western Blots**

Liver protein samples were homogenized using an extraction buffer (50 mM Tris, 1 mM EDTA, 1 mM EGTA, 10% glycerol, 1% Triton X-100 at pH 7.5 with 1 mM DTT, 1 mM PMSF, 5 ug/mL protease inhibitor cocktail, 10 ug/mL trypsin inhibitor, 50 mM NaF, and 5 mM NaPP added before homogenization). After homogenization, tissues were centrifuged for 10 min at 4° C. Protein extracts were separated by electrophoresis on an SDS-polyacrylamide gel (Invitrogen, Carlsbad, CA). Proteins were transferred to a PVDF transfer membrane (Bio-Rad, Hercules, CA) by electroblotting. Membranes were blocked with 5% nonfat dried milk in Tris-buffered saline-Tween (TBS-T) (Sigma, St. Louis, MO) and then incubated overnight with antibodies to total Akt, phospho-Akt (Ser473), β-actin (Cell Signaling, Danvers, MA), Binding immunoglobulin protein (BiP; Cell Signaling), C/EBP homologous protein (CHOP; Santa Cruz, Dallas, TX), and Phosphoenolpyruvate carboxykinase (PEPCK; Abcam, Cambridge, MA). After primary incubation, the blots were incubated with the appropriate peroxidase-conjugated secondary antibody (Cell Signaling) and analyzed using chemiluminescence (GE Healthcare, Piscataway, NJ). Bands were quantified via ImageJ software.

**Real Time PCR**
Total mRNA was extracted from liver, skeletal muscle, and brain using TRIzol. The cDNA was synthesized from 2 μg RNA with the High Capacity cDNA transcription kit. PCR amplification was carried out using the Bio-Rad CFX system with Taqman probes, and the ΔΔCt method was used to quantify mRNA levels. Data are represented using the Rq value, which is the normalization relative to the rtTA (control) group or Rq=2^{-ΔΔCt} [ΔCt=Ct (target)-Ct (GAPDH); ΔΔCt= ΔCt (sample)-ΔCt (rtTA)]. Gene expression was normalized against the expression of GAPDH. All PCR products were retrieved from Life Science Technologies (Grand Island, NY).

Glycogen phosphorylase and Glycogen synthase Assays

Liver samples were homogenized (~10 mg in 500 μL) in buffer (50 mM Tris:pH 6.8, 100 mM NaF, 10 mM EDTA, 5.0 mM dithioerythritol, and 0.5% glycogen). Glycogen synthase activity was measured after incubating the homogenate with the synthase reaction medium (50 mM Tris: pH 7.5, 1.5 mM UDP-glucose, 5.0 mM EDTA, 1% glycogen, 5.0 uCi [14C] UDP-glucose, and 3.0 mM glucose-6-phosphate). Glycogen phosphorylase activity was measured after incubating the homogenate with phosphorylase reaction medium (150 mM NaF, 15 mM glucose-1-phosphate, 1.5% glycogen, 5.0 uCi [14C]glucose-1-phosphate, and 7.5 mM AMP). Both reactions were incubated at 37°C, and 50 μL aliquots were spotted onto chromatography filter paper every 15 min for 30 min. The spotted filter paper was washed in 70% ethanol, and radioactivity was measured. Total glycogen synthase activity was calculated by the activity in the presence of 3.0 mM glucose-6-phosphate per mg of tissue. The activity ratio was calculated dividing the activity of glycogen synthase in the absence of glucose-6-
phosphate and by the activity in the presence of glucose-6-phosphate. Total glycogen phosphorylase activity was calculated as the activity in the presence of 7.5 mM AMP per mg of tissue. The activity of glycogen synthase represents the rate of glucose-6-phosphate incorporation into glycogen. The activity of glycogen phosphorylase is calculated as the rate of incorporation of glucose-1-phosphate into glycogen.

**Lactate Assay**

Plasma samples from the basal period of the clamp ($t=-10$ min & 0 min) were deproteinized with 4% perchloric acid. Lactate levels were determined in the supernatant by an enzymatic (lactate dehydrogenase) assay where the fluorescence of NADH was assessed on a 96 well plate fluorometer as previously described (26).

**Data Analysis**

Data are presented as the mean ± SEM. Paired comparisons were performed with the two-tailed Student’s $t$ test or ANOVA with repeated measures. The significance level was set at $p<0.05$.

**Results**

The activation NF-κB in the lung airway epithelium causes lung inflammation and systemic inflammation.

It has previously been demonstrated that the treatment of IKTA mice with Dox results in activation of NF-κB in the airway epithelium, and the consequent neutrophilic inflammation in the airways and alveoli (10). After 5 days of Dox, immune cell infiltration was increased in the IKTA mice (Figure 1 A&B). In IKTA mice, the total number of cells
in BAL fluid increased (Figure 1C), largely due to an increase in polymorphonuclear neutrophils (Figure 1D). Additionally, proinflammatory cytokines IL-5, TNF-α, and IL-6 were increased in the BAL fluid in the IKTA vs. control mice (Figure 1E). Lastly, circulating levels of these same cytokines were increased in the plasma of the IKTA mice (Figure 1F). IL-1β in both BAL and plasma was at or below the limit of detection (3.2 pg/mL; data not reported) in both groups.

Lung airway inflammation impairs the action of insulin in vivo

After the 5 days of Dox treatment, C57BL6/J background mice underwent hyperinsulinemic-euglycemic clamps to evaluate insulin action in vivo (Figure 2). During the clamp, the glucose levels were matched between groups (Figure 2A), but the glucose infusion rate (GIR) was lower in the IKTA group (Figure 2B). The decreased GIR indicates that the IKTA mice had decreased insulin sensitivity. This decrease occurred despite IKTA mice having higher plasma insulin concentrations during the clamp (1.72-fold higher than control; Figure 2C), which is consistent with insulin resistance.

Lung inflammation impairs hepatic insulin action and decreases peripheral tissue glucose uptake

The ability of insulin to enhance peripheral glucose uptake and to suppress endogenous glucose production was assessed in C57BL/6 background mice. Despite higher insulin levels in IKTA mice, the induction of lung inflammation in IKTA mice significantly decreased tissue glucose uptake ($R_a$) in gastrocnemius muscle and the brain. The $R_a$ (rate of appearance of glucose) was not significantly different between groups (Figure
Lastly, insulin's ability to suppress endogenous glucose production (Endo-Ra) was blunted in the IKTA mice (Figure 3C).

**Lung airway inflammation triggered systemic and liver inflammation**

Hypoxemia from airway inflammation could lead to systemic insulin resistance. Hypoxemia was not present as arterial PO$_2$ on the day of the clamp study was 93±9 vs. 101±5 mmHg in IKTA and control littermates, respectively. Airway inflammation, however, triggered systemic inflammation. The mRNA expression of *Il6* (IL-6), *Nos2* (iNOS) and *Tnf* (TNFα) in the brain, superficial vastus lateralis muscle, gastrocnemius muscle, and the liver were increased in the IKTA mice (Figures 4 & 5). In addition, the expression of CHOP protein was also increased (~2-fold) in the liver, which could indicate an increase in ER stress (Figure 5 B). However, another marker of ER stress, BiP was unchanged (Figure 5 B).

To understand the mechanism that accounts for the failure of insulin to suppress hepatic glucose production in the IKTA mice, we assessed markers of the insulin signaling pathway. The phosphorylation of Akt at Serine 473 was higher (~2.5-fold) in the IKTA mice (Figure 6A). Gene expression of downstream targets of insulin signaling, *Pck1* (PEPCK) and *G6pc* (G6Pase), were also assessed (Figure 6 B&C). Despite severe hepatic insulin resistance, the IKTA mice had a lower mRNA expression of *Pck1* and *G6pc*. In addition, PEPCK protein content was not different between the control and IKTA mice (Figure 6D). Glycogen synthase and phosphorylase activity was measured to determine if activity of these enzymes was different between groups. Insulin stimulates the activity of glycogen synthase and inhibits the activity glycogen phosphorylase. The
total activity of glycogen phosphorylase was not increased (p=0.07) in the IKTA mice (Fig. 7A), and the activity ratio of glycogen phosphorylase (0.83±0.03 vs. 0.85 +/- 0.02; control vs. IKTA) was not different between groups. Total activity (Fig. 7B) and the activity ratio (0.17 ± 0.02 vs. 0.12 +/- 0.02; control vs. IKTA) of glycogen synthase were similar between IKTA and control mice.

Given that increased glycogenolysis was not detected, we examined the factors important in regulating gluconeogenesis. While the expression of genes that control gluconeogenic capacity was not increased in the IKTA mice, circulating concentrations of the gluconeogenic substrate lactate was increased (Fig. 7C). Since increases in the amount of lactate in the plasma can increase gluconeogenesis, this could contribute to the persistent glucose production in the presence of insulin by the liver in the IKTA mice (13, 36, 48).

Discussion

Lung diseases are associated with an increased risk for development of type 2 diabetes, as they also increase known risk factors (e.g. obesity), the direct link between lung inflammation and glucose dyshomeostasis is unclear (6, 40, 44). This study highlights a direct link between airway inflammation and whole body insulin action. The aim of this study was to understand if localized airway inflammation, similar to what occurs during lung disease, has a negative impact on the insulin regulation of glucose metabolism. Previous work has correlated lung diseases with impaired insulin action but this link has not been directly tested. Additionally, these studies do not address if the inflammation
that occurs with many lung diseases impacts insulin action. We assessed the impact of airway specific inflammation on insulin action using a mouse model where we could chronically and selectively induce inflammation in the airway epithelium. Our findings show that targeted induction of low grade inflammation in the lung airway epithelium that does not result in systemic hypoxemia triggers systemic insulin resistance and inflammation in liver and peripheral tissues.

The model system that was used in these studies triggers lung airway inflammation specifically in the epithelium (10, 43). The activation of NF-κB in the lung airway not only triggers inflammation and the accumulation of immune cells in the lung, but also causes an increase in circulating plasma pro-inflammatory cytokines IL-6, IL-5 and TNF-α (Figure 1). While prior studies used a dose of Dox that induced enough airway injury to induce hypoxemia (10, 46), in the present study the dose and duration of doxycycline was optimized to avoid the confounding effects of hypoxemia.

Impairment in hepatic insulin action is the primary mechanism whereby airway inflammation impairs whole body insulin action in IKTA mice. The glucose requirements during the clamps for mice with inflammation in the airway epithelium were decreased by 23% in the IKTA mice relative to the control mice, despite higher insulin concentrations in the IKTA mice. Based on the calculated tracer-determined whole body glucose flux, the lower glucose requirements were primarily due to a failure of insulin to suppress hepatic glucose production (Fig 2C). In control mice, insulin completely suppressed hepatic glucose production. In contrast, hepatic glucose production in the IKTA mice was not suppressed despite higher insulin concentrations.
The inability of insulin to inhibit the production of glucose by the liver was associated with hepatic inflammation and systemic inflammation. Like other tissues, the liver had an increase in the expression of pro-inflammatory makers (Il6, Tnf, and Nos2), which could induce hepatic insulin resistance (21). In addition, circulating concentrations of TNFα, IL-5, and IL-6 were increased, which could also contribute to the observed hepatic insulin resistance. As has been reported with lung injury, IL-5 is increased and may explain the accumulation of polymorphonuclear neutrophils in the lung (15). The role of IL-5 in insulin action is unclear; a recent report suggests that the lack of IL-5 exacerbates insulin resistance when animals are on a high fat diet (32). In individuals with airway disease, systemic IL-6 is increased and may, along with increased TNF-α, contribute to an increased diabetes risk (16, 17, 21). In the current study, we used mice on a C57BL/6 background to evaluate insulin action as opposed to Balb/c, as they are prone to dietary and inflammation-induced insulin resistance, and have a robust cytokine response (2, 33, 35). However, it is possible that the absolute increase in plasma cytokines in response to airway injury may differ from that on a Balb/c background (46). The increase in inflammation in the liver could explain the insulin resistance. Insulin resistance is observed during systemic inflammatory events like obesity and endotoxemia, which also trigger an inflammatory response in the liver (7, 8, 18, 19, 24, 30). There are reports showing a crosstalk between the liver and the lung; although most of the prior work is focused on the impact of liver injury on lung inflammation (23). The present data suggest that the crosstalk is reciprocal; inflammation in the lung induces hepatic inflammation. Besides inducing inflammation in the liver, airway inflammation increased CHOP
expression. CHOP, which is commonly increased with ER stress, may contribute to the hepatic insulin resistance (27). However BiP, another ER stress marker, was not increased. Therefore, activation of ER stress alone would not be enough to explain the hepatic insulin resistance.

The insulin resistance that occurs due to inflammation typically results in suppression of the Akt signaling pathway (14, 18, 20, 49). It is not clear in vivo whether impaired insulin signaling in the liver via Akt is the contributor to insulin resistance. In support of the idea that Akt signaling does not always explain the insulin resistance phenotype, Akt phosphorylation and signaling in the liver was intact in IKTA mice. If hepatic insulin signaling were impaired due to systemic inflammation, there should be a blunted response to insulin on downstream gene targets as well as a blunted phosphorylation of Akt (25). However, the RNA expression of Pck2 and G6pc was not increased; in fact it was significantly lower in IKTA mice. Although mRNA expression of Pck2 was lower in the IKTA mice, protein levels of PEPCK were unchanged. In addition to gene targets, insulin also acts via the Akt signaling pathway to suppress the breakdown of glycogen by suppressing the activity of glycogen phosphorylase (25, 38). However, the activity of glycogen phosphorylase was not increased and trended lower in the mice with inflammation in their lung airway. Thus, insulin resistance in the liver cannot be explained by a failure of insulin to activate the traditional insulin-signaling pathway.

The increased production of glucose in the presence of insulin by the liver in IKTA mice could result from increased gluconeogenesis. As mentioned, the activities of glycogen synthase and phosphorylase between mice with and without inflammation of the lung
Airway cannot explain the failure of insulin to suppress hepatic glucose production. The protein expression of PEPCK was unchanged, however the availability of gluconeogenic substrates, e.g. lactate, was increased and could drive gluconeogenic flux (13, 36). This elevated lactate could enter the liver and drive an increase in glucose production through gluconeogenesis in spite of intact insulin signaling. It is unlikely the rise in lactate alone can explain the impaired suppression of hepatic glucose production. However, it may synergize with low grade hepatic inflammation.

Airway inflammation impaired glucose uptake into various peripheral tissues. Glucose uptake was significantly decreased in the gastrocnemius muscle and trended toward a decrease in the vastus lateralis and soleus muscle. White adipose tissue glucose uptake was unaffected. The expression of Il6 and Nos2 in the gastrocnemius and vastus muscles were increased, likely reflecting increases in muscle inflammation. These increases combined with the increase in circulating IL-6 and TNF-α, probably contribute to the decrease in muscle insulin action. Interestingly, we also observed a decrease in glucose uptake and increased expression of proinflammatory markers (Tnf, Nos2, and Il6) in the brain. Various reports indicate that decreases in blood flow and endothelial dysfunction can negatively impact glucose uptake into muscle tissue. It is possible that this could contribute to the decrease in the glucose uptake into the brain (4, 5, 20, 28, 39). It is also possible that inflammation in the lung airway epithelium could impact glucose homeostasis due to a negative effect on muscle blood flow and microvascular recruitment without necessarily impacting insulin signaling (11).
Perspectives and Significance

This study indicates that there is a direct link between low grade inflammation in the lung airway epithelium and systemic insulin resistance. Individuals with lung inflammation (COPD, asthma and pneumonia) are at greater risk of developing hyperglycemia or diabetes. While they have lung inflammation, they also have other associated issues that can cause insulin resistance (e.g. hypoxia, obesity or steroid treatment). Thus, it was not known whether primary airway inflammation without the accompanying comorbidities could cause systemic inflammation and insulin resistance. In our studies, we demonstrate that primary airway inflammation does cause systemic inflammation and insulin resistance. Moreover, the liver was a major site of the insulin resistance. Lung airway induced hepatic inflammation, suggesting crosstalk between the liver and the lung. Thus airway inflammation, even without the accompanying hypoxemia or obesity that can develop in various lung diseases, may contribute to the increased prevalence of insulin resistance and hyperglycemia in these individuals (3, 6, 9, 17, 29, 42, 47). Thus, new therapies that can limit airway inflammation to treat lung injury may have additional benefits beyond improving lung function. They may lower the risk of systemic inflammation and insulin resistance.

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Figure Legends

Figure 1: The induction of NF-κB dependent lung inflammation increases circulating cytokines in the plasma. (A) Lung section stained with H&E in control mice (BALB/c). (B) Lung section stained with H&E in IKTA mice. (C) Total number of cells in the BAL fluid. (D) Total number of macrophages, lymphocytes, and polymorphonuclear neutrophils in the BAL fluid. (E) Concentration of cytokines in the BAL fluid. (F) Concentration of cytokines in the plasma. The data represent the mean ± the SE. "*" indicates p<0.05.

Figure 2: Lung inflammation decreases the amount of glucose required to maintain euglycemia even though mice with lung inflammation have more circulating insulin during the clamp. The doxycycline-inducible mice (IKTA, n=11) and controls (TA, n=9) underwent a hyperinsulinemic-euglycemic clamp to measure whole-body insulin action after receiving doxycycline for 5 days (C57BL/6J). Mice were treated with doxycycline to induce NF-κB expression, induce pro-inflammatory cytokine production, and lung inflammation in IKTA mice. (A) Blood glucose concentrations during the clamp. (B) Glucose infusion rate during the clamp (GIR). (C) Plasma insulin concentrations during the basal period prior to the clamp and during the clamp. The data represent the mean ± the SE. "**" indicates p<0.05.

Figure 3: Lung inflammation decreases glucose uptake. Mice with lung inflammation are not able to shut off the endogenous production of glucose. (A) Tissue glucose uptake (Rg). (B) Rate of glucose appearance during the clamp (Ra). (C) Rate of endogenous
glucose production (Endo-Ra). The data represent the mean ± the SE. “**” indicates p<0.05.

Figure 4: Lung inflammation increases pro-inflammatory cytokines in peripheral tissues. The mRNA expression of markers for inflammation is induced by lung inflammation. (A) Expression of pro-inflammatory cytokines in brain tissue. (B) Expression of inflammatory cytokines in superficial vastus lateralis muscle. (C) Expression of pro-inflammatory cytokines in gastrocnemius muscle. The mRNA expression of il6, nos2, and tnf is corrected against the expression of gapdh (Rq). The data represent the mean ± the SE. “**” indicates p<0.05.

Figure 5: Liver inflammation and ER stress marker CHOP but not BiP are increased in mice with lung inflammation. The mRNA expression of markers for inflammation, and ER stress are all induced by lung inflammation. The mRNA expression of il6, nos2, and tnf is normalized against the expression of gapdh (Rq). (B) The protein expression of CHOP and BiP are normalized against ß-actin (A.U.). The data represent the mean ± the SE. “**” indicates p<0.05.

Figure 6: Insulin resistance in the liver is not explained by traditional insulin-induced signaling pathways. The insulin signaling pathway appears intact in the mice that have lung inflammation. Akt phosphorylation, pck2 expression, and g6pc expression were measured at the end of the clamp. (A) A western blot for Akt phosphorylation. (B) RNA expression levels of g6pc. (C) RNA expression levels of pck2. (D) A representative western blot for PEPCK protein. The amount of phosphorylated Akt is calculated as the
average of phosphorylated protein divided by total Akt protein (A.U.). The protein expression of PEPCK is normalized against β-actin (A.U.). The RNA expression of g6pc and pck2 is normalized against the expression of gapdh (Rq). The data represent the mean ± the SE. “*” indicates p<0.05.

Figure 7: The increased glucose production in the liver that results from lung inflammation does not arise from increased glycogen breakdown and is likely due to an increase in substrate supply to gluconeogenesis. (A) Glycogen phosphorylase activity was normalized to mg of tissue per reaction. (B) Glycogen synthase activity was normalized to mg of tissue per reaction. (C) Arterial Plasma lactate concentration. The data represent the mean ± the SE. “*” indicates p<0.05.
Figure 2

A. Blood Glucose (mg/dl) over time (min) for IKTA and Control groups.

B. GIR (mg/kg/min) over time (min) for IKTA and Control groups.

C. Plasma Insulin (ng/ml) at Basal and Clamp conditions for IKTA and Control groups.
Figure 4

A

B

C

* indicates a statistically significant difference.