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Deletion of interleukin-6 improves pyruvate tolerance without altering hepatic insulin signaling in the leptin receptor–deficient mouse

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ABSTRACT

Obesity is associated with increased circulating interleukin-6 (IL-6), which may contribute to hepatic insulin resistance by impairing insulin receptor signaling. This study was designed to assess the impact of the systemic absence of IL-6 on the development of insulin resistance and glucose intolerance in an obese mouse model. Systemic insulin, glucose, and pyruvate tolerance tests were performed in IL-6 knockout (IL6KO) mice that had been crossed with a genetically obese (leptin receptor–deficient mouse model [Lep^{db}]) mouse model. Real-time reverse transcriptase polymerase chain reaction and Western blot analysis assessed cellular and molecular markers of insulin signaling, inflammation, and metabolism. Absence of IL-6 did not improve systemic glucose or insulin tolerance, but Lep^{db} × IL6KO mice displayed a smaller blood glucose increase following a pyruvate challenge. These results suggest that loss of IL-6 in the context of obesity may locally reduce hepatic glucose production from a gluconeogenic precursor. Hepatic insulin-dependent insulin receptor autophosphorylation, Akt activation, and FoxO1 phosphorylation were similar between Lep^{db} × IL6KO mice and Lep^{db} controls. Basal gene expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase was reduced in male Lep^{db} × IL6KO mice relative to Lep^{db} controls; but gene expression of another regulatory enzyme, glucose-6-phosphatase, remained unaltered. Absence of IL-6 reduced gene expression of serum amyloid A and RelA in female Lep^{db} mice, but did not alter hepatic triglyceride accumulation or lipogenic gene expression. Overall, our results suggest that IL-6 may be detrimental in obesity by contributing to elevated hepatic glucose output.

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1. Introduction

Interleukin-6 (IL-6) is a pleiotropic cytokine whose role in metabolism remains controversial. Circulating IL-6 is positively correlated with development of obesity and insulin resistance [1,2]. Our laboratory has demonstrated that chronic IL-6 impairs insulin receptor autophosphorylation, insulin receptor substrate-1 phosphorylation, and Akt activation in

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hepatocytes [3,4]. This effect is partially mediated through the induction of suppressor of cytokine signaling 3 (SOCS3) [5]. Others have shown that SOCS3 can impair insulin signaling in muscle and adipose tissue [6-8]. In contrast, Sadagurski et al [9] demonstrated that overexpression of IL-6 induces hepatic SOCS3, but does not alter insulin signaling.

Effects of IL-6 in the muscle are also conflicting. Acute, muscle-derived IL-6 can locally enhance insulin sensitivity during exercise [10,11]. This effect appears to be mediated through activation of adenosine monophosphate–activated protein kinase (AMPK), which promotes β-oxidation and lipid clearance [12,13]. Despite these reports, the majority of exercise-induced AMPK activation was preserved in IL-6 knockout (IL6KO) mice [14]. This latter finding would argue that IL-6 may not be the primary effector of exercise-induced AMPK activation and enhanced insulin action.

These conflicting roles for IL-6 may be explained by a dose and time dependency. Supraphysiologic doses are required to elicit the beneficial metabolic effects of IL-6 [15] and are 10 to 35 times higher than elevations observed in obesity-mediated insulin resistance [16,17]. Nieto-Vasquez and colleagues [18] effectively demonstrated that whereas acute IL-6 can activate AMPK and enhance muscle insulin sensitivity, chronic IL-6 exposure activates protein-tyrosine phosphatase 1B, SOCS3, and c-Jun N-terminal kinase, which impair insulin signaling.

The IL6KO mouse itself has yielded conflicting conclusions about the metabolic role of IL-6. An initial study by Wallenius et al [19] demonstrated that IL6KO mice develop mature-onset obesity and impaired glucose tolerance beginning at around 6 months of age. Recently, Matthews et al [20] reported similar effects in approximately 20-week-old mice. In contrast, Chida et al [21] and Di Gregorio et al [22] did not observe any change in body mass or adiposity in IL6KO mice compared with wild-type controls at 16 weeks or 3 and 14 months, respectively. Neither group observed changes in fasted glucose metabolism. Similarly, high-fat diet–fed IL6KO mice have yielded contrasting results [20,22,23].

Leptin-deficient (Lep^{ob}) and leptin-resistant (Lep^{db}) mice are hyperphagic, obese, and insulin resistant [24]. We previously reported that neutralization of IL-6 in Lep^{ob} mice improved systemic insulin responsiveness and hepatic insulin signaling [25]. In addition, antisense-mediated knockdown of SOCS3 improved insulin signaling in Lep^{db} mice [26]. Here we crossed Lep^{db} and IL6KO mice (Lep^{db} × IL6KO) to assess the impact of chronic IL-6 absence on development of inflammation and insulin resistance of obesity.

2. Materials and methods

2.1. *Generation of Lep^{db} × IL6KO mice*

Leptin receptor–deficient mice (B6.BKS[D]-$Lepr^{db}$/J, stock 697) were bred with IL6KO mice (B6.129S2-Il6<tm1Kopf>/J, stock 2650) by Jackson Laboratory Services (Bar Harbor, ME, USA) and shipped to the University of Rochester. These breeder pairs were heterozygous for $Lepr^{db}$ (Lep^{db}/+) and homozygous for Il6<tm1Kopf> (IL6KO), which produced IL6KO offspring that were either lean or obese. Control animals were obtained through heterozygous breeding of the Lep^{db}/+ parent strain (B6.BKS[D]-$Lepr^{db}$/J, stock 697), which produced IL6+ offspring that were either lean or obese. Both parent lines had undergone extensive backcrossing (≥12 times) onto the B6 strain before the special mating, which resulted in animals with greater than 99.98% B6 genetic homology. As a result, influence of their initial genetic background strain is assumed to be negligible. All animals were bred and housed in a microisolator room on a 12-hour light/dark cycle at the University of Rochester. Mating of Lep^{db}/+ × IL6KO male and female mice yielded smaller litter size compared with Lep^{db}/+ × IL6+ controls (average pups per litter: 4.4 ± 0.23, IL6KO vs 5.6 ± 0.24, IL6+; $P = .0003$) but maintained appropriate mendelian inheritance patterns. Offspring were separated by sex; and littermates were housed less than or equal to 5 per cage, depending on litter size. Animals were allowed free, unmonitored access to a standard chow diet (4.14 kcal/g gross energy; 28.8% from protein, 12.7% from fat, and 58.5% from carbohydrate) (Laboratory Autoclavable Rodent Diet 5010; LabDiet, Richmond, IN). Experiments were performed at an average age of 17.7 weeks (±0.3 weeks). The University Committee on Animal Resources approved all protocols.

2.2. *Metabolic studies*

Briefly, mice were fasted overnight and given an intraperitoneal injection of insulin (1.0 U/kg, lean; 1.5 U/kg, obese),

Table 1 – Characterization of fasted male mice

	Lean (+/+)		Obese (Lep^{db})		
	IL-6+	IL6KO	IL-6+	IL6KO	
Body weight (g)	21.9 ± 0.8	22.8 ± 0.9	46.9 ± 1.7 *	43.0 ± 1.6 *,†	n ≥ 14
Glucose (mg/dL)	87.1 ± 6.5	99.6 ± 9.9	135.9 ± 15.0 *	151.5 ± 13.4 *	n ≥ 11
Epididymal WAT weight (% body weight)	0.67 ± 0.08	0.74 ± 0.10	4.82 ± 0.11 *	4.46 ± 0.33 *	n = 3
Liver weight (% body weight)	3.8 ± 0.3	3.3 ± 0.1	4.6 ± 0.2 *	4.7 ± 0.2 *	n = 3
Liver glycogen (mg glycogen/mg protein)	n/a	n/a	1.3 ± 0.4	1.6 ± 0.2	n = 3
Liver triglyceride (mg triglyceride/mg protein)	0.76 ± 0.17	0.22 ± 0.06 †	1.80 ± 0.22 *	1.39 ± 0.15 *	n = 3

Glycogen and triglyceride contents (milligrams per wet weight) were normalized to protein content (milligrams per wet weight). Data represent the mean ± SE. WAT indicates white adipose tissue.

* $P \leq .01$ compared with respective lean control.

† $P \leq .05$ compared with respective IL-6+ control.

Table 2 – Characterization of fasted female mice

	Lean (+/+)		Obese (Lep^{db})		
	IL-6+	IL6KO	IL-6+	IL6KO	
Body weight (g)	19.9 ± 0.6	20.6 ± 0.8	50.6 ± 2.1†	46.3 ± 2.1†	n ≥ 13
Glucose (mg/dL)	71.3 ± 1.8	76.8 ± 6.9	153.9 ± 16.0†	124.2 ± 11.2*	n ≥ 13
HOMA-IR	2.6 ± 2.1	8.5 ± 4.5	62.9 ± 25.1*	54.3 ± 15.5	n ≥ 3
Liver weight (% body weight)	3.4 ± 0.1	3.2 ± 0.1	4.7 ± 0.2†	4.5 ± 0.2†	n ≥ 5
Liver glycogen (mg glycogen/mg protein)	0.7 ± 0.2	1.3 ± 0.2	1.7 ± 0.2	1.9 ± 0.5	n ≥ 4
Liver triglyceride (mg triglyceride/mg protein)	0.40 ± 0.06	0.17 ± 0.02‡	0.96 ± 0.07†	0.73 ± 0.15†	n ≥ 4

HOMA-IR indicates homeostatic model assessment of insulin resistance and was calculated as follows: [fasted insulin (microunits per milliliter) × fasted glucose (millimoles per liter)]/22.5. Glycogen and triglyceride contents (milligrams per wet weight) were normalized to protein content (milligrams per wet weight). Data represent the mean ± SE.

* $P \leq .05$ compared with respective lean control.

† $P \leq .01$ compared with respective lean control.

‡ $P \leq .05$ compared with respective IL-6+ control.

glucose (1.5 g/kg), or sodium pyruvate (2.0 g/kg) dissolved in sterile saline. Blood glucose was measured from tail vein every 15 minutes using an Accu-chek Advantage glucometer (Roche Diagnostics, Indianapolis, IN, USA). Each set of animals was used in 2 of 3 sequential metabolic tests with at least 1 week between experiments. Data presented for each test are the result of 2 independent experiments. Area under the curve (AUC) for the pyruvate tolerance test (PTT) was calculated using blood glucose values relative to the average of the lean IL6+ animal values.

2.3. *Assessment of in vivo insulin signaling*

Animals were fasted overnight (~15 hours), briefly anesthetized using an isoflurane vaporizer (Summit Medical, Bend, OR), and given an intraperitoneal injection of vehicle (sterile saline) or human insulin (Novolin; Novo Nordisk Inc., Princeton, NJ, USA) (1.5 U/kg, lean; 2.5 U/kg, obese). Lep^{db} mice received a higher insulin dose to achieve detectable, but submaximal insulin signaling response. After 10 minutes, animals were briefly anesthetized and euthanized by cervical dislocation before tissue extraction. Tissues were immediately excised and snap frozen in liquid nitrogen. Whole cell lysates were extracted from frozen tissue via homogenization in lysis buffer (100 mmol/L HEPES [pH 7.4], 150 mmol/L NaCl, 1% Triton X-100, 10% glycerol, 2 mmol/L EDTA, 2 mmol/L EGTA, protease inhibitor cocktail [Calbiochem, La Jolla, CA], 1 mmol/L phenylmethylsulfonyl fluoride, 10 mmol/L benzamidine, 10 mmol/L tetrasodium pyrophosphate, and 5 mmol/L activated sodium orthovanadate) and centrifugation at 20 000*g*. Protein was quantified using the Bradford method [27]. Fresh lysate was probed with insulin receptor β chain antibody for 2 hours at 4°C, followed by incubation with protein A beads for an additional 1 hour at 4°C. Beads were washed and deproteinated with 1× Laemmli buffer before gel electrophoresis. Western blot assessments were performed by running lysate or immunoprecipitate on a polyacrylamide gel, transferring to nitrocellulose membrane, probing for target proteins with primary and secondary (horseradish peroxidase–conjugated) antibodies, and developing via chemiluminescence. Graphs represent the results of 2 independent experiments.

2.4. *Antibodies and insulin measurement*

Cell Signaling Technology (Danvers, MA, USA) antibodies used were as follows: phospho-specific Akt (Ser473), phospho-specific signal transducer and activator of transcription 3 (STAT3) (Tyr705), STAT3 mass, and fatty acid synthase (FAS) mass. Santa Cruz Biotechnology (Santa Cruz, CA, USA) antibodies used were as follows: Akt1/2, β-actin, and insulin receptor β chain. Anti-phosphotyrosine antibody was purchased from Millipore (Billerica, MA, USA). Fasted insulin levels were determined using the Ultra Sensitive Mouse Insulin ELISA Kit from Crystal Chem (Downers Grove, IL).

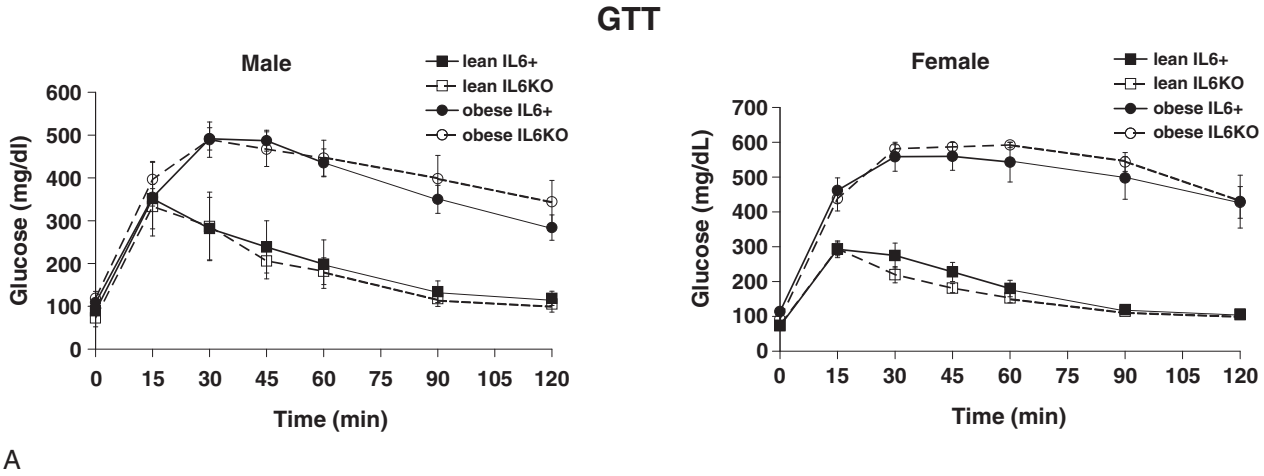
2.5. *Lipid extraction and analysis*

The lipid extraction protocol was adapted from Burant et al [28]. Frozen liver was weighed and homogenized in chloroform-methanol (2:1 vol/vol). Extracts were passed through fluted filter paper. Sulfuric acid (0.05% in saline) was added to filtered extract at a ratio of 1:5 (vol/vol). Following centrifugation, the chloroform layer was removed, dried down, and resuspended in fresh chloroform. Samples were diluted in 5% Triton X-100 (Sigma Chemical, St. Louis, MO, USA) (in chloroform) and evaporated. Lipids were measured in duplicate using L-Type Tg kit from Wako Chemicals (Richmond, VA).

2.6. *Glycogen extraction and analysis*

The liver glycogen extraction protocol was adapted from Shen et al [29]. Liver pieces (0.1-0.2 g) were digested in 1.0 mL of 30%

Fig. 1 – Metabolic challenge tests in Lep^{db} × IL6KO mice. Male and female Wt × IL-6+ (lean IL6+), Wt × IL6KO (lean IL6KO), Lep^{db} × IL-6+ (obese IL6+), and Lep^{db} × IL6KO (obese IL6KO) were fasted overnight. Blood glucose was monitored following intraperitoneal administration of (A) 1.5 g/kg glucose, (B) 1.0 U/kg (lean) and 1.5 U/kg (obese) insulin, or (C) 2.0 g/kg pyruvate. Total AUC is expressed as mean ± SE relative to average lean IL6+ values. Lean males, n ≥ 5; obese males, n ≥ 4; lean females, n ≥ 6; and obese females, n = 7. *$P \leq .05$; †$P \leq .01$.


GTT
Male
Female
lean IL6+
lean IL6KO
obese IL6+
obese IL6KO
Glucose (mg/dl)
Glucose (mg/dL)
Time (min)
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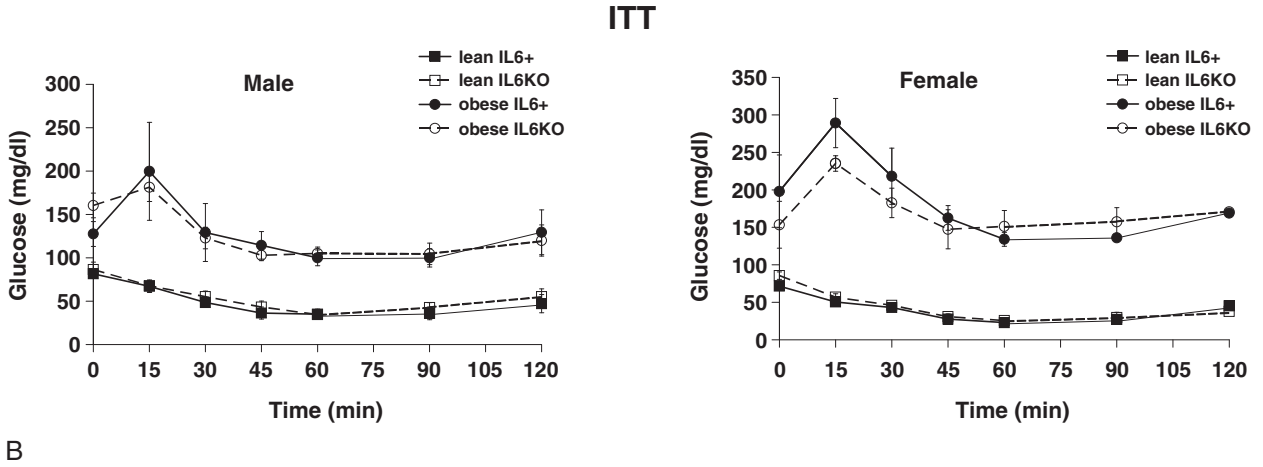
ITT
Male
Female
lean IL6+
lean IL6KO
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obese IL6KO
Glucose (mg/dl)
Glucose (mg/dL)
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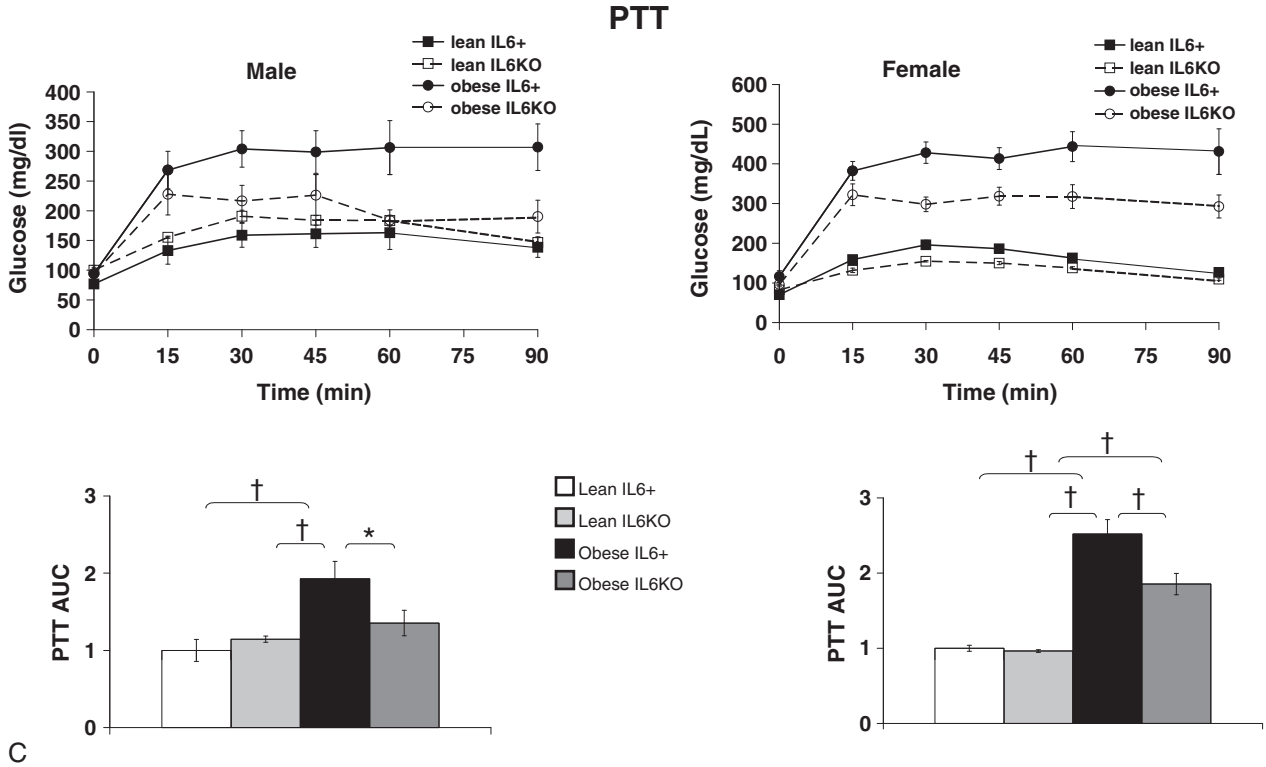
PTT
Male
Female
lean IL6+
lean IL6KO
obese IL6+
obese IL6KO
Glucose (mg/dl)
Glucose (mg/dL)
Time (min)
PTT AUC
Lean IL6+
Lean IL6KO
Obese IL6+
Obese IL6KO
C

KOH at 95°C for 30 minutes. A total of 1.5 mL of 95% EtOH was added, and samples were spun at 3000*g* for 20 minutes. The glycogen pellet was washed with water and 95% ethanol, and dissolved in 0.5 mL of water. For quantification, 5 μL of sample was added to 14.6 mmol/L anthrone reagent (Sigma) and incubated at 90°C for 20 minutes. Absorbance was read at 620 nm against a glucose standard curve.

2.7. *Real-time polymerase chain reaction analysis*

RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's directions. An iSCRIPT kit (Bio-Rad, Hercules, CA, USA) was used for reverse transcription. SYBR Green (Bio-Rad) reactions were performed on an iCycler IQ real-time polymerase chain reaction (PCR) detection system (Bio-Rad), and calculations were determined as previously described [25]. All target genes were normalized to acidic ribosomal phosphoprotein P0 (36B4) housekeeping gene. Primer sequences are available upon request.

2.8. *Statistical analysis*

Statistical analysis was performed using StatView 5 software (SAS Institute, Cary, NC). One-way analysis of variance was used to compare sample means among 4 groups, and Fisher protected least significant difference test was used to determine between-group significance. When statistically appropriate, interquartile range calculations were used to remove statistical outliers from individual assays; however, no complete data set for any experimental animal was excluded from the entire study based on outlier analysis.

3. Results

3.1. *Absence of IL-6 in obesity does not alter glucose or insulin tolerance, but improves pyruvate tolerance*

As expected, body weight of male (Table 1) and female (Table 2) Lep^{db} mice was significantly higher than that of lean controls. Whereas absence of IL-6 reduced body weight of male Lep^{db} mice compared with IL6+ controls, epididymal fat mass was not significantly different (Table 1). Loss of IL-6 did not alter fasted glucose levels in male (Table 1) or female (Table 2) Lep^{db} × IL6KO mice compared with Lep^{db} × IL6+ controls. The homeostatic model assessment of insulin resistance, a measure of insulin resistance, was elevated in female Lep^{db} mice compared with lean controls, but unaltered by the absence of IL-6 in either model (Table 2). Whereas Lep^{db} mice displayed impairment of glucose tolerance, absence of IL-6 did not alter response to a glucose bolus in male or female obese mice (Fig. 1A). In addition, impaired insulin tolerance in Lep^{db} mice was not affected by loss of IL-6 (Fig. 1B). These results suggest that absence of IL-6 cannot overcome obesity-mediated impairment of systemic insulin action in this animal model. Absence of IL-6 had no effect on glucose or insulin tolerance in lean mice.

To more directly assess the role of IL-6 in hepatic glucose homeostasis in obesity, mice were challenged with the gluconeogenic precursor pyruvate. The ability of the PTT to reflect changes in hepatic glucose production has been demonstrated by several groups [30-32]. Absence of IL-6 improved pyruvate tolerance in male and female Lep^{db} mice as represented by a significant reduction in AUC (Fig. 1C). Given that systemic glucose and insulin tolerance were unaltered, this effect suggests reduced hepatic glucose output in the absence of IL-6.

3.2. *Absence of IL-6 does not alter early insulin signaling in liver of Lep^{db} mice*

Downstream insulin signaling effects are mediated by Akt activation, including impairment of gluconeogenesis by inhibitory phosphorylation of the transcription factor FOXO1. The phosphorylation state of insulin signaling molecules was assessed by Western blot analysis in fasted animals following a 10-minute insulin bolus. Although insulin-stimulated lean and obese animals cannot be directly compared because of different insulin boluses, absence of IL-6 did not alter hepatic insulin-dependent insulin receptor autophosphorylation or Akt serine phosphorylation in lean or Lep^{db} mice (Fig. 2A). Insulin-stimulated phosphorylation of FOXO1 was unaltered in lean IL6KO mice compared with IL6+ controls (Fig. 2A). A modest elevation in basal FOXO1 phosphorylation reflects previous observations in Lep^{db} mice [33], but insulin-stimulated phosphorylation was unaffected by the absence of IL-6 (Fig. 2A).

Fasting hepatic expression of *Pck* (phosphoenolpyruvate carboxykinase) and *G6pc* (glucose-6-phosphatase [G6Pase]) was analyzed to assess whether loss of IL-6 alters basal gluconeogenic gene expression in Lep^{db} mice. Expression of *Pck* was reduced by 40% in male Lep^{db} × IL6KO mice (Fig. 2B), but *G6pc* remained unaltered by the absence of IL-6 in male and female obese mice (Fig. 2B). Glycogen metabolism also regulates blood glucose concentration, but absence of IL-6 did not alter basal hepatic glycogen content in male (Table 1) or female (Table 2) Lep^{db} mice. These results indicate that absence of IL-6 improves pyruvate tolerance in Lep^{db} × IL6KO mice, but this may not be due to direct effects of IL-6 on glucose metabolism.

3.3. *Unaltered lipogenesis in Lep^{db} × IL6KO mice*

Increased lipogenesis may promote conversion and storage of pyruvate as triglyceride and indirectly decrease glucose output in Lep^{db} × IL6KO mice. To assess activity of this pathway, hepatic markers of lipogenesis were examined. Consistent with genetic obesity, FAS abundance was elevated in Lep^{db} liver (Fig. 3A). *Scd1* (stearoyl-CoA desaturase 1) expression was also increased in obese mice compared with lean controls (Fig. 3B), which is likely associated with absence of leptin signaling [34]. Expression of *Srebf1* (sterol regulatory element binding protein [SREBP]–1c) was significantly increased in female Lep^{db} mice compared with lean controls (Fig. 3B). There was no effect, however, of the absence of IL-6 on these markers. Quantitation of liver triglyceride was lower in lean IL6KO mice compared with IL6+ controls, but systemic absence of IL-6 did not alter hepatic steatosis in male (Table 1) or female (Table 2) Lep^{db} mice.

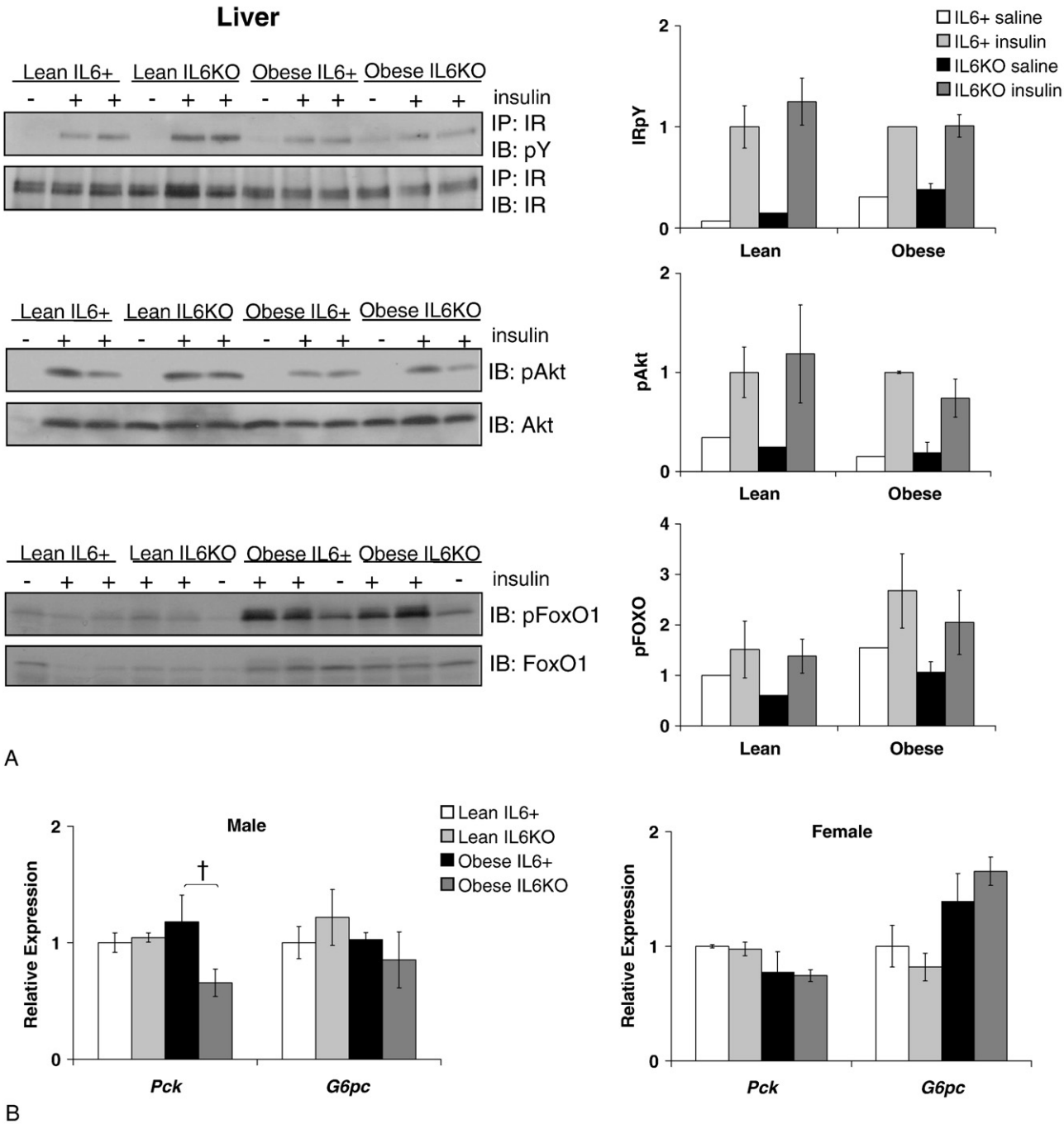

Fig. 2 – Insulin signaling and gluconeogenic gene expression in Lep^{db} × IL6KO mice. Male Wt × IL-6+ (lean IL6+), Wt × IL6KO (lean IL6KO), Lep^{db} IL-6+ (obese IL6+), and Lep^{db} × IL6KO (obese IL6KO) were fasted overnight. A, Following a 10-minute intraperitoneal insulin bolus (1.5 U/kg, lean; 2.5 U/kg, obese), hepatic insulin receptor was immunoprecipitated (IP); and tyrosine autophosphorylation was assessed by Western blot (IB) analysis. Hepatic Akt (Ser 473) phosphorylation in whole cell lysates and FOXO1 (Ser256) phosphorylation in cytoplasmic fractions were also detected by Western blot analysis following insulin administration. Graphs represent quantitation of densitometric values relative to the average insulin-stimulated, IL6+ animals (IRpY and pAkt) or average basal, lean IL6+ animals (pFOXO). No significant differences were detected between insulin-stimulated, IL6+ and IL6KO animals. Basal state, n ≥ 2; insulin stimulated, n ≥ 3. B, Expression of phosphoenolpyruvate carboxykinase (*Pck*) and G6Pase (*G6pc*) was assessed in RNA from livers of male and female mice by real-time reverse transcriptase (RT)–PCR and expressed as mean ± SE relative to average lean IL6+ values. Lean, n ≥ 5; obese, n ≥ 4. $^{\dagger}P \leq .01$.

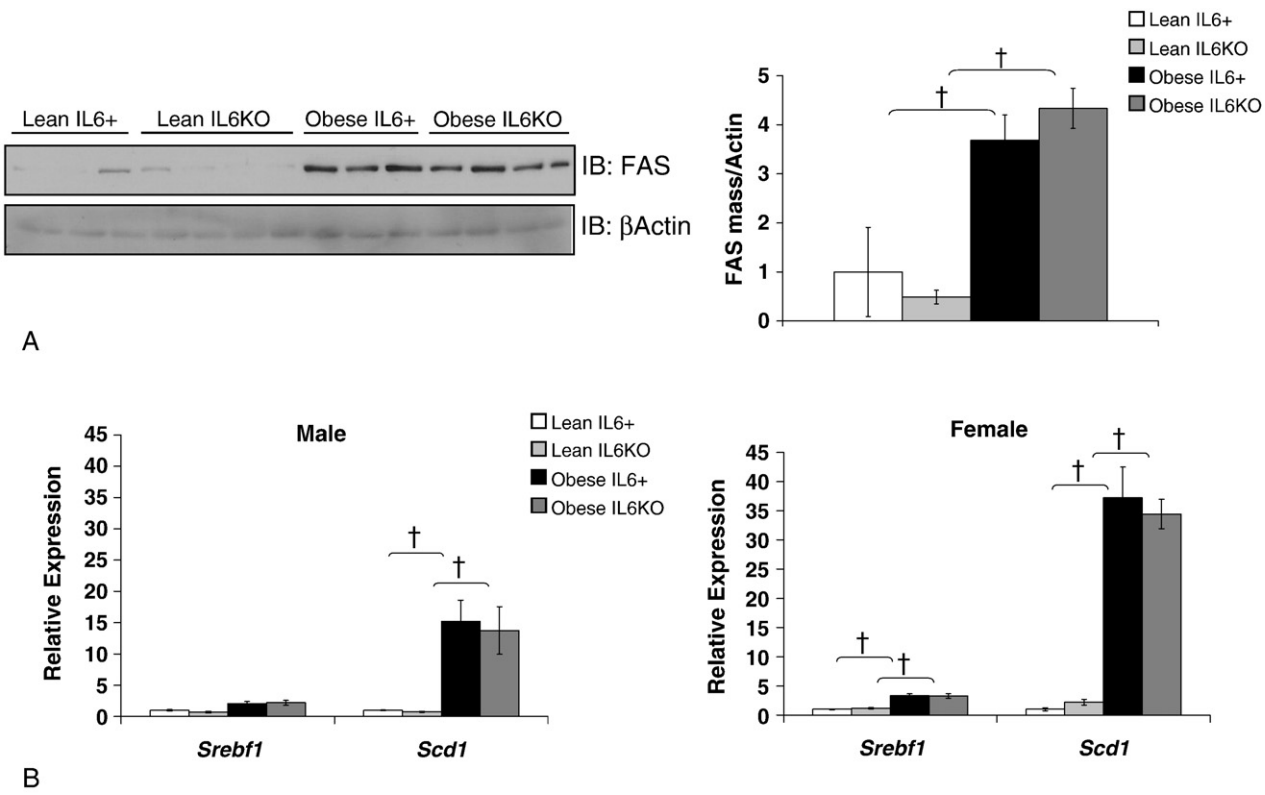

Fig. 3 – Hepatic lipogenic markers in Lep^{db} × IL6KO mice. Liver was harvested from male and female Wt × IL-6+ (lean IL6+), Wt × IL6KO (lean IL6KO), Lep^{db} IL-6+ (obese IL6+), and Lep^{db} × IL6KO (obese IL6KO) mice following an overnight fast. A, Hepatic FAS abundance was determined by Western blot analysis in female mice. B, Expression of the lipogenic genes SREBP-1c (*Srebf1*) and SCD1 (*Scd1*) was assessed in RNA from livers of male and female mice by real-time RT-PCR and expressed as mean ± SE relative to average lean IL6+ values. Lean, n ≥ 5; obese, n ≥ 4. $^{\dagger}P \leq .01$.

3.4. *Absence of IL-6 results in modest reduction of hepatic inflammation in Lep^{db} mice*

To further understand how loss of IL-6 could impact the local hepatic inflammatory environment, STAT3 phosphorylation (Tyr-705) and target gene induction were assessed. Hepatic phosphorylation of STAT3 was significantly elevated in Lep^{db} mice compared with lean mice (Fig. 4A). This corresponded with increased gene expression of the acute phase protein serum amyloid A (SAA) in female Lep^{db} mice compared with lean controls, with a similar trend in male mice (Fig. 4B). Although STAT3 phosphorylation trended downward in IL6KO mice compared with IL6+ controls (Fig. 4A), a significant reduction in *Saa1* gene expression in female Lep^{db} × IL6KO mice (Fig. 4B) suggested that induction of the acute phase response in this obese model may be partially attenuated by absence of IL-6.

IκB kinase β/nuclear factor κB (NFκB)–mediated signaling is associated with impaired hepatic metabolism [35]. Hepatic expression of the NFκB transcription factor component *Rela* was reduced in female Lep^{db} × IL6KO mice by 27% compared with IL6+ controls; however, this effect was not observed in male mice (Fig. 4B).

4. Discussion

The present study confirms previous reports [21,22] that IL6KO mice do not develop obesity or insulin resistance compared with wild-type controls. This is in contrast to the observations of Wallenius et al [19] and Matthews et al [20]. Although Wallenius et al [19] did not observe changes in weight or glucose tolerance before 6 months of age, the more recent study by Matthews et al [20] reported significant weight gain in conjunction with impaired glucose and insulin tolerance at 20 weeks old. Although the reasons for the observed differences are unclear, the potential development of mature-onset obesity and metabolic changes could be affected by differences in dietary nutritional balance, regional background strain variation, and housing strategy. In fact, despite being only 2 weeks older than the mice used in the current study, the wild-type and IL6KO mice used by Matthews et al weighed approximately 10 and 15 g more, respectively. This remarkable difference in body mass could have an important impact on metabolic parameters and, thus, experimental conclusions.

Absence of IL-6 did not improve systemic glucose and insulin tolerance in the genetically obese Lep^{db} mouse model. Similar observations have been made in diet-induced obese (DIO) IL6KO mice [20,22,23]. These reports indicate similar weight gain and fasted glucose levels accompanied by modest elevation in blood glucose levels during a glucose tolerance test in DIO IL6KO mice compared with DIO IL6+ controls. In these models, it is feasible that, in the absence of IL-6, other factors associated with diet-induced obesity continue to suppress insulin sensitivity [36]. In contrast to these studies, Wunderlich et al [37] reported that hepatocyte-specific loss of

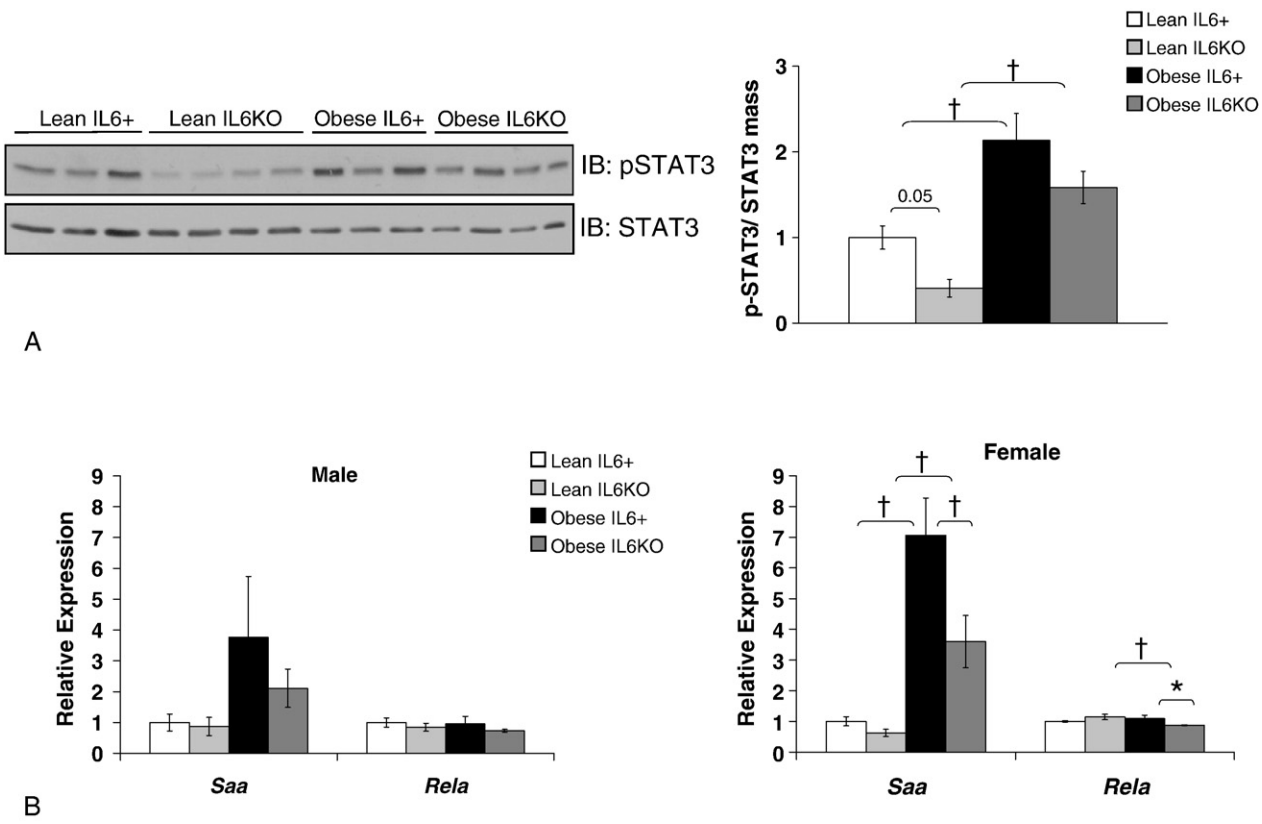

Fig. 4 – Assessment of hepatic STAT3-responsive signaling. Liver was harvested from male and female Wt × IL-6+ (lean IL6+), Wt × IL6KO (lean IL6KO), Lepdb IL-6+ (obese IL6+), and Lepdb × IL6KO (obese IL6KO) mice following an overnight fast. A, Hepatic STAT3 (Tyr 705) phosphorylation and abundance were determined by Western blot analysis in female mice. B, Serum amyloid A (*Saa*) and RelA (*Rela*) gene expression was assessed by real-time RT-PCR of RNA from livers of male and female mice. Gene expression data are expressed as mean ± SE relative to average lean IL6+ values. Lean, n ≥ 5; obese, n ≥ 4. $^{*}P \leq .05$; $^{\dagger}P \leq .01$.

IL-6 signaling impaired glucose and insulin tolerance in the basal state of lean animals, suggesting that IL-6 is required for physiologic maintenance of glucose homeostasis. The absence of a similar effect in our mouse model could reflect the difference between chronic, systemic absence of a signaling molecule and tissue-specific signaling inactivation. In addition, a hyperinsulinemic-euglycemic clamp elicited a strong inflammatory response in the study of Wunderlich et al [37]. Under these conditions, absence of IL-6 signaling in hepatocytes reduced systemic insulin-stimulated glucose transport, indicating that IL-6 may preserve insulin sensitivity during acute inflammation. As a potentially important anti-inflammatory role of IL-6 was only observed during the clamp, it is unclear whether similar effects would be observed in the Lep^{db} × IL6KO mouse under the same conditions.

Whereas we explored the contribution of IL-6 to the obese, insulin-resistant phenotype of the Lep^{db} mouse model, Sadagurski et al [9] examined the potential benefit of IL-6 overexpression on energy expenditure in the Lep^{ob} mouse model. Although IL-6 overexpression dramatically protected against high-fat diet–induced obesity and insulin resistance, it was unable to fully prevent development of the obese, insulin-resistant phenotype in the Lep^{ob} mouse despite increased central leptin sensitivity. When combined with the currently reported results, the data indicate that chronic manipulation of IL-6 in the context of leptin deficiency or resistance does not alter development of obesity or systemic insulin resistance. Adding to the complexity, Chida et al [21] observed that combined IL-1 and IL-6 deficiency results in modest weight gain, whereas single knockout controls remained lean. As IL-1 signaling has been implicated in central leptin action [38], this result suggests a synergistic, central effect of these 2 molecules. The independent central effect(s) of IL-6 and the complicated cross talk between leptin, IL-1, and IL-6, however, have yet to be fully elucidated.

The current study demonstrated that absence of IL-6 in Lep^{db} mice leads to smaller increases in circulating glucose levels in response to a pyruvate bolus compared with IL-6+ controls. As the glucose tolerance test and insulin tolerance test did not indicate a systemic change in glucose utilization as a function of IL-6, the response to pyruvate is likely due to a reduction in hepatic utilization of pyruvate for the production of circulating glucose. Interleukin-6 could control glucose output directly by altering glucose metabolism or indirectly through regulation of other metabolic pathways that require pyruvate as a substrate. Based on our observations, the effect of IL-6 deletion cannot be accounted for by changes in insulin receptor signaling or suppression of gluconeogenic gene expression because *Pck* expression was decreased only in males. This does not completely rule out gluconeogenic

control, however, as Samuel et al [39] observed increased endogenous glucose production via gluconeogenesis in diabetic rats and humans independent of changes in *Pck* and *G6pc* expression. In light of this report, it remains possible that allosteric regulation of fructose-1,6-bisphosphatase by F2,6P2 [40] and subcellular localization of G6Pase [41] are potential posttranscriptional regulatory targets for IL-6.

Interleukin-6 has been reported to suppress insulin-mediated glycogen synthesis [3,42] and directly stimulate hepatic glycogenolysis [43]. Thus, removal of IL-6 could promote glycogen synthesis and/or reduce basal glycogenolysis, thereby reducing hepatic glucose output. Interestingly, Wunderlich et al [37] demonstrated that rendering hepatocytes unresponsive to IL-6 did not alter basal hepatic glucose metabolism or glycogen content, but increased hepatic glycogen synthesis during a hyperinsulinemic-euglycemic clamp. The former result is similar to our observation that basal glycogen content was similar in Lep^{db} × IL6KO and IL6+ controls. A hyperinsulinemic-euglycemic clamp and radiolabeled glucose infusion may be required to more sensitively detect potential differences in glucose metabolism in our model.

In addition to direct effects on glucose metabolism, loss of IL-6 could enhance activity of other pyruvate-consuming pathways at the expense of substrate availability for gluconeogenesis. We explored the possibility that Lep^{db} × IL6KO mice display increased lipogenesis. Absence of IL-6 did not alter abundance of FAS or expression of SREBP-1c, the master regulator of lipogenesis [44]. In addition, basal hepatic triglyceride accumulation was similar in Lep^{db} × IL6+ and Lep^{db} × IL6KO mice. It remains possible that IL-6 could alter pyruvate flux by regulating pyruvate dehydrogenase activity, altering lactic acid formation, or modulating cellular respiration/mitochondrial consumption of pyruvate in Lep^{db} mice. Interestingly, Matthews et al [20] observed altered hepatic mitochondrial function in DIO IL6KO mice in association with hepatic inflammatory infiltrates and reduced insulin-stimulated Akt activation. Given that pyruvate carboxylase is localized to the mitochondria and required for the utilization of pyruvate in gluconeogenesis [45], it could be hypothesized that altered hepatic mitochondrial function in the Lep^{db} × IL6KO model would result in decreased glucose production during the PTT.

Absence of systemic IL-6 in female Lep^{db} mice modestly reduced markers of hepatic inflammation, including transcription of *Saa1* and *Rela*. Given that low-grade activation of the acute phase response is associated with increased hepatic glucose output [46-48], absence of IL-6 may blunt activation of the acute phase response and subsequently reduce hepatic use of pyruvate for glucose production. Inhibition of NFκB-mediated inflammation has also been associated with restored suppression of hepatic gluconeogenesis and reduced glucose production from pyruvate in Lep^{db} mice [49]. A modest reduction in expression of *Rela* in female Lep^{db} × IL6KO mice is consistent with this latter effect.

In summary, absence of IL-6 in Lep^{db} mice improved pyruvate tolerance in association with modest reduction in hepatic inflammation but no apparent improvement in insulin receptor signaling. This study provides further support for a contributory role of IL-6 to metabolic dysregulation in the Lep^{db} mouse model, but further studies will be required to define the precise mechanism of IL-6 action.

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Conflict of Interest

The authors have no conflict of interest to report.

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