Published in final edited form as:

Metabolism. 2011 November ; 60(11): 1610–1619. doi:10.1016/j.metabol.2011.04.004.

Deletion of interleukin-6 improves pyruvate tolerance in the leptin receptor-deficient mouse

Alicia H. Clementi^a, Allison M. Gaudy^b, Teresa A. Zimmers^{c,d}, Leonidas G. Koniaris^{c,d}, and Robert A. Mooney^{a,*}

^a Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, NY

^b Department of Pharmacology and Physiology, University of Rochester Medical Center

^c Department of Cell Biology and Anatomy, University of Miami Miller School of Medicine, Miami FL

^d Department of Surgery, University of Miami Miller School of Medicine, Miami, Florida

Abstract

Objective—Obesity is associated with increased circulating interleukin-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.

Materials/Methods—Systemic insulin, glucose, and pyruvate tolerance tests were performed in IL-6 knockout (IL6KO) mice that had been crossed with a genetically obese (Lep^{db}) mouse model. Real-time RT-PCR and Western blot analysis assessed cellular and molecular markers of insulin signaling, inflammation, and metabolism.

Results—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, PEPCK, was reduced in male Lep^{db} × IL6KO mice relative to Lep^{db} controls, but gene expression of another regulatory enzyme, G6Pase, 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.

© 2011 Elsevier Inc. All rights reserved.

*Corresponding author: Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, 601 Elmwood Ave., Rochester, NY 14642, robert_mooney@urmc.rochester.edu, Tel: 585-275-7811 Fax: 585-756-4468.

Disclosure Statement

The authors have no conflicts of interest to report.

Author Contributions

A.H.C. designed the study, performed experiments, and prepared the manuscript; A.M.G. performed experiments; T.A.Z. and L.G.K. provided preliminary data and mice; R.A.M. designed the study and prepared the manuscript. All authors read and approved the manuscript.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conclusion—Overall, our results suggest that IL-6 may be detrimental in obesity by contributing to elevated hepatic glucose output.

Keywords

obesity; leptin; inflammation; insulin resistance; liver

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 lab has demonstrated that chronic IL-6 impairs insulin receptor autophosphorylation, insulin receptor substrate-1 phosphorylation, and Akt activation in 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 over-expression 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 AMP-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 data 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 10–35 times higher than elevations observed in obesity-mediated insulin resistance [16, 17]. Nieto-Vasquez and colleagues [18] effectively demonstrated that while acute IL-6 can activate AMPK and enhance muscle insulin sensitivity, chronic IL-6 exposure activates PTP1B, SOCS3 and JNK, 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 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 to 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]. Additionally, antisense-mediated knockdown of SOCS3 improved insulin signaling in Lep^{db} mice [26]. Here we crossed Lep^{db} and IL6KO mice ($Lep^{db} \times IL6KO$) to assess the impact of chronic IL-6 absence on development of inflammation and insulin resistance of obesity.

MATERIALS AND METHODS

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 and shipped to the University of Rochester. These breeder pairs were heterozygous for Lepr^{db} (Lepr^{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 Lepr^{db/+} parent strain (B6.BKS(D)-Lepr^{db}/J, stock #697), which produced IL6+ offspring that either were lean or obese. Both parent lines had undergone extensive backcrossing (≥12 times) onto the B6 strain prior to the special mating, which resulted in animals with >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 hr light/dark cycle at the University of Rochester. Mating of Lepr^{db/+} × IL6KO male and female mice yielded smaller litter size compared to Lepr^{db/+} × IL6+ controls (avg. pups/litter 4.4 ± 0.23, IL6KO vs 5.6 ± 0.24, IL6+; p=0.0003) but maintained appropriate Mendelian inheritance patterns. Offspring were separated by sex and littermates were housed ≤5 per cage, depending on litter size. Animals were allowed free, unmonitored access to a standard chow diet (4.14 kcal/gm 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.

Metabolic studies

Briefly, mice were fasted overnight and given an intraperitoneal injection of insulin (1.0U/kg, lean; 1.5U/kg, obese), glucose (1.5g/kg), or sodium pyruvate (2.0g/kg) dissolved in sterile saline. Blood glucose was measured from tail vein every 15 min using an Accu-chek Advantage® glucometer (Roche). Each set of animals was used in two of three sequential metabolic tests with ≥1 week between experiments. Data presented for each test are the result of two independent experiments. Area under the curve for the pyruvate tolerance test was calculated using blood glucose values relative to the average of the lean IL6+ animal values.

Assessment of *in vivo* insulin signaling

Animals were fasted overnight (~15 hr), briefly anesthetized using an isoflurane vaporizer (Summit Medical), and given an intraperitoneal injection of vehicle (sterile saline) or human insulin (Novolin®, Novo Nordisk Pharmaceuticals) (1.5U/kg, lean; 2.5U/kg, obese). Lep^{db} mice received a higher insulin dose to achieve detectable, but sub-maximal insulin signaling response. After 10 minutes, animals were briefly anesthetized and euthanized by cervical dislocation prior to 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 mM HEPES (pH 7.4), 150 mM NaCl, 1% Triton X-100, 10% glycerol, 2mM EDTA, 2mM EGTA, protease inhibitor cocktail (Calbiochem, La Jolla, CA), 1 mM phenylmethylsulfonylfluoride, 10mMbenzamidine, 10mmtetrasodiumpyrophosphate, and 5mM activated sodium orthovanadate) and centrifugation at 20,000 × g. Protein was quantified using the Bradford method [27]. Fresh lysate was probed with insulin receptor beta chain antibody for 2hr. 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 prior to 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 (HRP-conjugated antibodies), and

developing via chemiluminescence. Graphs represent the results of two independent experiments.

Antibodies and insulin measurement

Cell Signaling Technology antibodies: phospho-specific Akt (Ser473), phospho-specific STAT3 (Tyr705), STAT3 mass, and FAS mass. Santa Cruz Biotechnology antibodies: Akt1/2, β -actin, and insulin receptor beta chain. Anti-phosphotyrosine antibody was purchased from Millipore. Fasted insulin levels were determined using the Ultra Sensitive Mouse Insulin ELISA Kit from Crystal Chem (Downers Grove, IL).

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 v/v). Extracts were passed through fluted filter paper. Sulfuric acid (0.05% in saline) was added to filtered extract at a ratio of 1:5 (v/v). Following centrifugation, the chloroform layer was removed, dried down and resuspended in fresh chloroform. Samples were diluted in 5% Triton X-100 (Sigma) (in chloroform) and evaporated. Lipids were measured in duplicate using L-Type Tg kit from (Wako Chemicals).

Glycogen extraction and analysis

The liver glycogen extraction protocol was adapted from Shen et al. [29]. Liver pieces (0.1–0.2g) were digested in 1.0ml of 30% KOH at 95°C for 30 min. 1.5ml of 95% EtOH was added and samples spun at 3000 $\times$ g for 20 min. The glycogen pellet was washed with water and 95% ethanol, and dissolved in 0.5ml of water. For quantification, 5 μ l of sample was added to 14.6mM anthrone reagent (Sigma), incubated at 90°C for 20 minutes. Absorbance was read at 620nm against a glucose standard curve.

Real-Time PCR Analysis

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

Statistical analysis

Statistical analysis was performed using StatView 5 software (SAS Institute). One-way ANOVA was used to compare sample means among four groups and Fischer's 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.

RESULTS

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 lean controls. While absence of IL-6 reduced body weight of male *Lep^{db}* mice compared to 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) $Lep^{db} \times IL6KO$ mice compared to $Lep^{db} \times IL6+$ controls. HOMA-IR, a measure of insulin resistance, was elevated in female Lep^{db} mice compared to lean controls, but unaltered by the absence of IL-6 in either model (Table 2). While 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). Additionally, 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 pyruvate tolerance test 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 area under the curve (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.

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 min 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 to $IL6+$ 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 absence of IL-6 (Fig. 2A).

Fasting hepatic expression of *Pck* (PEPCK) and *G6pc* (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 $Lep^{db} \times IL6KO$ 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 $Lep^{db} \times IL6KO$ mice, but this may not be due to direct effects of IL-6 on glucose metabolism.

Unaltered lipogenesis in $Lep^{db} \times IL6KO$ mice

Increased lipogenesis may promote conversion and storage of pyruvate as triglyceride and indirectly decrease glucose output in $Lep^{db} \times IL6KO$ mice. To assess activity of this pathway, hepatic markers of lipogenesis were examined. Consistent with genetic obesity, fatty acid synthase (FAS) abundance was elevated in Lep^{db} liver (Fig. 3A). *Scd1* (SCD-1) expression was also increased in obese mice compared to lean controls (Fig. 3B), which is likely associated with absence of leptin signaling [34]. Expression of *Srebf1* (SREBP-1c) was significantly increased in female Lep^{db} mice compared to 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 to $IL6+$ controls, but systemic absence of IL-6 did not alter hepatic steatosis in male (Table 1) or female (Table 2) Lep^{db} mice.

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 to leans (Fig. 4A). This corresponded with increased gene expression of the acute phase protein serum amyloid A (SAA) in female *Lep^{db}* mice compared to lean controls, with a similar trend in males (Fig. 4B). While STAT3 phosphorylation trended downward in IL6KO mice compared to 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.

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

DISCUSSION

The present study confirms previous reports [21, 22] that IL6KO mice do not develop obesity or insulin resistance compared to 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. While 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 IL-6KO mice used by Matthews et al. weighed approximately 10g and 15g 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 to 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. reported that hepatocyte-specific loss of 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 [37]. 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. Additionally, a hyperinsulinemic-euglycemic clamp elicited a strong inflammatory response in the Wunderlich study [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}* × IL-6KO mouse under the same conditions.

While 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 over-

expression on energy expenditure in the *Lep^{ob}* mouse model. Although IL-6 over-expression 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, while single knock-out controls remained lean. As IL-1 signaling has been implicated in central leptin action [38], this result suggests a synergistic, central effect of these two 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 to IL-6+ controls. As the GTT and ITT 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. IL-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 since *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 (FBPase) by F2,6P2 [40] and subcellular localization of G6Pase [41] are potential post-transcriptional regulatory targets for IL-6.

IL-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 *Leb^{db} × IL6KO* and IL6+ controls. A hyperinsulinemic-euglycemic clamp and radiolabelled 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]. Additionally, 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 $Lep^{db} \times IL6KO$ model would result in decreased glucose production during the pyruvate tolerance test.

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 usage of pyruvate for glucose production. Inhibition of NF κ B-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 $Lep^{db} \times IL6KO$ 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.

Acknowledgments

Funding

This work was funded in part by NIH RO1-DK060732 to R.A.M.

Abbreviations

AMPK	AMP-activated protein kinase
FAS	fatty acid synthase
G6Pase	glucose-6-phosphatase
GTT	glucose tolerance test
HOMA-IR	homeostatic model assessment of insulin resistance
IL-6	interleukin-6
IL6KO	IL-6 knock-out mouse model
ITT	insulin tolerance test
IKKβ	I κ B kinase beta
JNK	c-Jun N-terminal kinase
Lep^{db}	leptin receptor deficient mouse model
Lep^{ob}	leptin deficient mouse model
NFκB	nuclear factor kappa B
PEPCK	phosphoenolpyruvate carboxykinase
PTP1B	protein-tyrosine phosphatase 1B
PTT	pyruvate tolerance test
SAA	serum amyloid A
SCD-1	stearoyl-CoA desaturase 1
SOCS3	suppressor of cytokine signaling 3

SREBP-1c	sterol regulatory element binding protein
STAT3	signal transducer and activator of transcription 3

References

1. Pickup JC, Chusney GD, Thomas SM, Burt D. Plasma interleukin-6, tumour necrosis factor alpha and blood cytokine production in type 2 diabetes. *Life Sci.* 2000; 67(3):291–300. [PubMed: 10983873]
2. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA.* 2001; 286(3):327–34. [PubMed: 11466099]
3. Klover PJ, Zimmers TA, Koniaris LG, Mooney RA. Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. *Diabetes.* 2003; 52(11):2784–9. [PubMed: 14578297]
4. Senn JJ, Klover PJ, Nowak IA, Mooney RA. Interleukin-6 induces cellular insulin resistance in hepatocytes. *Diabetes.* 2002; 51(12):3391–9. [PubMed: 12453891]
5. Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW, Mooney RA. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem.* 2003; 278(16):13740–6. [PubMed: 12560330]
6. Emanuelli B, Peraldi P, Filloux C, Chavey C, Freidinger K, Hilton DJ, Hotamisligil GS, Van Obberghen E. SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-alpha in the adipose tissue of obese mice. *J Biol Chem.* 2001; 276(51):47944–9. [PubMed: 11604392]
7. Rieusset J, Bouzakri K, Chevillotte E, Ricard N, Jacquet D, Bastard JP, Laville M, Vidal H. Suppressor of cytokine signaling 3 expression and insulin resistance in skeletal muscle of obese and type 2 diabetic patients. *Diabetes.* 2004; 53(9):2232–41. [PubMed: 15331532]
8. Ueki K, Kondo T, Kahn CR. Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Mol Cell Biol.* 2004; 24(12):5434–46. [PubMed: 15169905]
9. Sadagurski M, Norquay L, Farhang J, D'Aquino K, Copps K, White MF. Human IL6 enhances leptin action in mice. *Diabetologia.* 2010; 53(3):525–35. [PubMed: 19902173]
10. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, Pedersen BK. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. *Diabetes.* 2004; 53(7):1643–8. [PubMed: 15220185]
11. Weigert C, Hennige AM, Brodbeck K, Haring HU, Schleicher ED. Interleukin-6 acts as insulin sensitizer on glycogen synthesis in human skeletal muscle cells by phosphorylation of Ser473 of Akt. *Am J Physiol Endocrinol Metab.* 2005; 289(2):E251–7. [PubMed: 15755769]
12. Al-Khalili L, Bouzakri K, Glund S, Lonnqvist F, Koistinen HA, Krook A. Signaling specificity of interleukin-6 action on glucose and lipid metabolism in skeletal muscle. *Mol Endocrinol.* 2006; 20(12):3364–75. [PubMed: 16945991]
13. Carey AL, Steinberg GR, Macaulay SL, et al. Interleukin-6 increases insulin-stimulated glucose disposal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. *Diabetes.* 2006; 55(10):2688–97. [PubMed: 17003332]
14. Kelly M, Keller C, Avilucea PR, et al. AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. *Biochem Biophys Res Commun.* 2004; 320(2):449–54. [PubMed: 15219849]
15. Geiger PC, Hancock C, Wright DC, Han DH, Holloszy JO. IL-6 increases muscle insulin sensitivity only at superphysiological levels. *Am J Physiol Endocrinol Metab.* 2007; 292(6):E1842–6. [PubMed: 17327367]
16. Ostrowski K, Rohde T, Zacho M, Asp S, Pedersen BK. Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *J Physiol.* 1998; 508 (Pt 3):949–53. [PubMed: 9518745]

17. Penkowa M, Keller C, Keller P, Jauffred S, Pedersen BK. Immunohistochemical detection of interleukin-6 in human skeletal muscle fibers following exercise. *FASEB J*. 2003; 17(14):2166–8. [PubMed: 12958150]
18. Nieto-Vazquez I, Fernandez-Veledo S, de Alvaro C, Lorenzo M. Dual role of interleukin-6 in regulating insulin sensitivity in murine skeletal muscle. *Diabetes*. 2008; 57(12):3211–21. [PubMed: 18796617]
19. Wallenius V, Wallenius K, Ahren B, Rudling M, Carlsten H, Dickson SL, Ohlsson C, Jansson JO. Interleukin-6-deficient mice develop mature-onset obesity. *Nat Med*. 2002; 8(1):75–9. [PubMed: 11786910]
20. Matthews VB, Allen TL, Risis S, et al. Interleukin-6-deficient mice develop hepatic inflammation and systemic insulin resistance. *Diabetologia*. 2010; 53(11):2431–41. [PubMed: 20697689]
21. Chida D, Osaka T, Hashimoto O, Iwakura Y. Combined interleukin-6 and interleukin-1 deficiency causes obesity in young mice. *Diabetes*. 2006; 55(4):971–7. [PubMed: 16567518]
22. Di Gregorio GB, Hensley L, Lu T, Ranganathan G, Kern PA. Lipid and carbohydrate metabolism in mice with a targeted mutation in the IL-6 gene: absence of development of age-related obesity. *Am J Physiol Endocrinol Metab*. 2004; 287(1):E182–7. [PubMed: 15191885]
23. Ellingsgaard H, Ehres JA, Hammar EB, et al. Interleukin-6 regulates pancreatic alpha-cell mass expansion. *Proc Natl Acad Sci U S A*. 2008; 105(35):13163–8. [PubMed: 18719127]
24. Coleman DL. Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia*. 1978; 14(3):141–8. [PubMed: 350680]
25. Klover PJ, Clementi AH, Mooney RA. Interleukin-6 depletion selectively improves hepatic insulin action in obesity. *Endocrinology*. 2005; 146(8):3417–27. [PubMed: 15845623]
26. Ueki K, Kondo T, Tseng YH, Kahn CR. Central role of suppressors of cytokine signaling proteins in hepatic steatosis, insulin resistance, and the metabolic syndrome in the mouse. *Proc Natl Acad Sci U S A*. 2004; 101(28):10422–7. [PubMed: 15240880]
27. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976; 72:248–54. [PubMed: 942051]
28. Burant CF, Sreenan S, Hirano K, et al. Troglitazone action is independent of adipose tissue. *J Clin Invest*. 1997; 100(11):2900–8. [PubMed: 9389757]
29. Shen W, Searce LM, Brestelli JE, Sund NJ, Kaestner KH. Foxa3 (hepatocyte nuclear factor 3gamma) is required for the regulation of hepatic GLUT2 expression and the maintenance of glucose homeostasis during a prolonged fast. *J Biol Chem*. 2001; 276(46):42812–7. [PubMed: 11546810]
30. Rodgers JT, Puigserver P. Fasting-dependent glucose and lipid metabolic response through hepatic sirtuin 1. *Proc Natl Acad Sci U S A*. 2007; 104(31):12861–6. [PubMed: 17646659]
31. Miyake K, Ogawa W, Matsumoto M, Nakamura T, Sakaue H, Kasuga M. Hyperinsulinemia, glucose intolerance, and dyslipidemia induced by acute inhibition of phosphoinositide 3-kinase signaling in the liver. *J Clin Invest*. 2002; 110(10):1483–91. [PubMed: 12438446]
32. Lin J, Wu PH, Tarr PT, et al. Defects in adaptive energy metabolism with CNS-linked hyperactivity in PGC-1alpha null mice. *Cell*. 2004; 119(1):121–35. [PubMed: 15454086]
33. Aoyama H, Daitoku H, Fukamizu A. Nutrient control of phosphorylation and translocation of Foxo1 in C57BL/6 and db/db mice. *Int J Mol Med*. 2006; 18(3):433–9. [PubMed: 16865227]
34. Cohen P, Miyazaki M, Socci ND, et al. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science*. 2002; 297(5579):240–3. [PubMed: 12114623]
35. Cai D, Yuan M, Frantz DF, Melendez PA, Hansen L, Lee J, Shoelson SE. Local and systemic insulin resistance resulting from hepatic activation of IKK-beta and NF-kappaB. *Nat Med*. 2005; 11(2):183–90. [PubMed: 15685173]
36. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr*. 2004; 92(3):347–55. [PubMed: 15469638]
37. Wunderlich FT, Strohle P, Konner AC, et al. Interleukin-6 signaling in liver-parenchymal cells suppresses hepatic inflammation and improves systemic insulin action. *Cell Metab*. 2010; 12(3):237–49. [PubMed: 20816090]

38. Luheshi GN, Gardner JD, Rushforth DA, Loudon AS, Rothwell NJ. Leptin actions on food intake and body temperature are mediated by IL-1. *Proc Natl Acad Sci U S A*. 1999; 96(12):7047–52. [PubMed: 10359836]
39. Samuel VT, Beddow SA, Iwasaki T, Zhang XM, Chu X, Still CD, Gerhard GS, Shulman GI. Fasting hyperglycemia is not associated with increased expression of PEPCK or G6Pc in patients with Type 2 Diabetes. *Proc Natl Acad Sci U S A*. 2009; 106(29):12121–6. [PubMed: 19587243]
40. Pilkis SJ, Granner DK. Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu Rev Physiol*. 1992; 54:885–909. [PubMed: 1562196]
41. van Schaftingen E, Gerin I. The glucose-6-phosphatase system. *Biochem J*. 2002; 362(Pt 3):513–32. [PubMed: 11879177]
42. Kanemaki T, Kitade H, Kaibori M, Sakitani K, Hiramatsu Y, Kamiyama Y, Ito S, Okumura T. Interleukin 1beta and interleukin 6, but not tumor necrosis factor alpha, inhibit insulin-stimulated glycogen synthesis in rat hepatocytes. *Hepatology*. 1998; 27(5):1296–303. [PubMed: 9581683]
43. Ritchie DG. Interleukin 6 stimulates hepatic glucose release from prelabeled glycogen pools. *Am J Physiol*. 1990; 258(1 Pt 1):E57–64. [PubMed: 2105658]
44. Shimano H, Yahagi N, Amemiya-Kudo M, et al. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. *J Biol Chem*. 1999; 274(50):35832–9. [PubMed: 10585467]
45. Jitrapakdee S, Wallace JC. Structure, function and regulation of pyruvate carboxylase. *Biochem J*. 1999; 340 (Pt 1):1–16. [PubMed: 10229653]
46. Festa A, D'Agostino R Jr, Howard G, Mykkanen L, Tracy RP, Haffner SM. Chronic subclinical inflammation as part of the insulin resistance syndrome: the Insulin Resistance Atherosclerosis Study (IRAS). *Circulation*. 2000; 102(1):42–7. [PubMed: 10880413]
47. Zhang J, Underwood LE, D'Ercole AJ. Hepatic mRNAs up-regulated by starvation: an expression profile determined by suppression subtractive hybridization. *FASEB J*. 2001; 15(7):1261–3. [PubMed: 11344107]
48. Simons JP, Schols AM, Buurman WA, Wouters EF. Weight loss and low body cell mass in males with lung cancer: relationship with systemic inflammation, acute-phase response, resting energy expenditure, and catabolic and anabolic hormones. *Clin Sci (Lond)*. 1999; 97(2):215–23. [PubMed: 10409477]
49. Tamura Y, Ogiwara T, Uchida T, et al. Amelioration of glucose tolerance by hepatic inhibition of nuclear factor kappaB in db/db mice. *Diabetologia*. 2007; 50(1):131–41. [PubMed: 17093946]

Figure 1. Metabolic challenge tests in $Lep^{db} \times IL6KO$ mice
Male and female $Wt \times IL-6+$ (lean $IL6+$), $Wt \times IL-6KO$ (lean $IL6KO$), $Lep^{db} \times IL-6+$ (obese $IL6+$), and $Lep^{db} \times IL-6KO$ (obese $IL6KO$) were fasted overnight. Blood glucose was monitored following intraperitoneal administration of **A**) 1.5g/kg glucose **B**) 1.0U/kg (lean) and 1.5U/kg (obese) insulin or **C**) 2.0g/kg pyruvate. Total AUC is expressed as mean $\pm$ S.E. relative to average lean $IL6+$ values. Lean males $n \geq 5$, obese males $n \geq 4$, lean females $n \geq 6$, and obese females $n = 7$. * $p \geq 0.05$; † $p \geq 0.01$

Figure 2. Insulin signaling and gluconeogenic gene expression in $Lep^{db} \times IL6KO$ mice
 Male $Wt \times IL-6+$ (lean $IL6+$), $Wt \times IL-6KO$ (lean $IL6KO$), $Lep^{db} IL-6+$ (obese $IL6+$), and $Lep^{db} \times IL-6KO$ (obese $IL6KO$) were fasted overnight. **A)** Following a 10 min intraperitoneal insulin bolus (1.5U/kg, lean; 2.5U/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 $n \geq 2$, insulin stimulated ≥ 3 . **B)** Expression of PEPCCK (*Pck*) and G6Pase (*G6pc*) was assessed in RNA from livers of male and female mice by real-time RT-PCR and expressed as mean $\pm$ S.E. relative to average lean $IL6+$ values. Lean $n \geq 5$, obese $n \geq 4$. $\dagger p \geq 0.01$

Figure 3. Hepatic lipogenic markers in $Lep^{db} \times IL6KO$ mice

Liver was harvested from male and female $Wt \times IL-6+$ (lean $IL6+$), $Wt \times IL-6KO$ (lean $IL6KO$), $Lep^{db} IL-6+$ (obese $IL6+$), and $Lep^{db} \times IL-6KO$ (obese $IL6KO$) mice following an overnight fast. **A)** Hepatic fatty acid synthase (FAS) abundance was determined by Western blot analysis in female mice. **B)** Expression of the lipogenic genes SREBP1c (*Srebf1*) and SCD1 (*Scd1*) was assessed in RNA from livers of male and female mice by real-time RT-PCR and expressed as mean $\pm$ S.E. relative to average lean $IL6+$ values. Lean $n \geq 5$, obese $n \geq 4$. $\dagger p \leq 0.01$

Figure 4. Assessment of hepatic STAT3-responsive signaling

Liver was harvested from male and female Wt $\times$ IL-6+ (lean IL6+), Wt $\times$ IL-6KO (lean IL6KO), Lepdb IL-6+ (obese IL6+), and Lepdb $\times$ IL-6KO (obese IL6KO) mice following an overnight fast. **A)** Hepatic STAT3 (Tyr 705) phosphorylation and abundance was determined by Western blot analysis in female mice. **B)** SAA (*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 $\pm$ S.E. relative to average lean IL6+ values. Lean $n \geq 5$; obese $n \geq 4$. * $p \leq 0.05$; † $p \leq 0.01$

TABLE 1

Characterization of fasted male mice

	Lean (+/+)		Obese (<i>Lep^{db}</i>)	
	IL-6+	IL-6KO	IL-6+	IL-6KO
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
Epi. 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 content (mg/wet weight) were normalized to protein content (mg/wet weight). Epi. WAT, Epididymal white adipose tissue. Data represent the mean ± S.E.

[†] p ≤ 0.01 compared to respective lean control.

[‡] p ≤ 0.05 compared to respective IL-6+ control.

Characterization of fasted female mice

TABLE 2

	Lean (+/+)		Obese (<i>Lep^{db}</i>)	
	IL-6+	IL-6KO	IL-6+	IL-6KO
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 was calculated: [fasted insulin (uU/ml) × fasted glucose (mmol/L)]/22.5. Glycogen and triglyceride content (mg/wet weight) were normalized to protein content (mg/wet weight). Data represent the mean ± S.E.

* p≤ 0.05,
[†] p ≤ 0.01 compared to respective lean control.
[‡] p ≤ 0.05 compared to respective IL-6+ control.