Chronic Exposure to Interleukin-6 Causes Hepatic Insulin Resistance in Mice

Peter J. Klover,[1,2] Teresa A. Zimmers,[3] Leonidas G. Koniaris,[3] and Robert A. Mooney[1]

Interleukin (IL)-6 is one of several proinflammatory cytokines associated with the insulin resistance of obesity and type 2 diabetes. There is, however, little direct evidence in vivo for a causative role of IL-6 in insulin resistance. Here, a 5-day constant subcutaneous infusion of hIL-6 before portal vein insulin challenge resulted in impairment of early insulin receptor signaling in the liver of mice. Importantly, the sixfold elevation of IL-6 attained with constant infusion was similar to levels reached in obesity. Consistent with an hepatic response to IL-6, STAT3 phosphorylation was increased in livers of IL-6–treated mice at 5 days. Chronic infusion of IL-6 also reduced hepatic insulin receptor autophosphorylation by 60% and tyrosine phosphorylation of insulin receptor substrates-1 and -2 by 60 and 40%, respectively. IL-6 had no effect on the mass of these proteins. IL-6 also decreased refeeding-dependent glucokinase mRNA induction by ~40%. Insulin tolerance tests revealed reduced insulin sensitivity. In contrast to hepatic insulin receptor signal transduction, 5-day IL-6 exposure failed to suppress skeletal muscle insulin receptor signal transduction. These data suggest that chronic IL-6 treatment selectively impairs hepatic insulin signaling in vivo, further supporting a role for IL-6 in hepatic insulin resistance of obesity. *Diabetes* 52: 2784–2789, 2003

Accumulating evidence suggests a link between inflammation and type 2 diabetes. Markers of inflammation, including C-reactive protein (CRP) and the proinflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin (IL)-1α and -1β, interferon-γ, and IL-6 are elevated in insulin-resistant patients with cancer, infection, trauma, and cachexia (1–5). Importantly, a recent study demonstrates that levels of CRP and IL-6 correlate with both insulin resistance and obesity and predict the development of type 2 diabetes (6).

It has been known for decades that obesity is an important risk factor for developing insulin resistance and type 2 diabetes. Therefore, an intriguing hypothesis is that secretion of endocrine factors by adipose tissue impairs insulin sensitivity and alters glucose metabolism. Indeed, studies have shown that the adipose tissue secretes IL-6, and of all the proinflammatory cytokines, circulating levels of IL-6 correlate most strongly with adiposity and type 2 diabetes (6,7). The liver is a likely target of IL-6 produced by adipose tissue. Omental fat is more strongly linked to insulin resistance than nonvisceral fat depots (8) and has been found to secrete as much as 2- to 3-fold more IL-6 than subcutaneous fat (9). Intriguingly, venous drainage of omental fat uses the portal venous system, hence potentially having a preferential effect on the liver.

Several studies have suggested that IL-6 can inhibit hepatic insulin signaling in vitro and in vivo. Recent studies by our group demonstrate that HepG2 cells and primary mouse hepatocytes show impaired insulin receptor signaling and insulin-dependent glycogen synthesis when acutely pretreated with IL-6 (10). In vivo studies have shown that IL-6 has a detrimental effect on glucose homeostasis. In rodents, IL-6 injection leads to increased plasma glucose and insulin levels after 90 min (11). In normal volunteers, IL-6 administration at clinically relevant levels increases fasting glucose levels when measured 1 h after treatment (12). Additionally, we have reported that mice acutely exposed to IL-6 for 90 min have reduced insulin signal transduction in the liver (13). These results are all consistent with the hypothesis that IL-6 impairs insulin sensitivity, and the liver may be an important target of IL-6 produced by the adipose tissue.

In a physiological context, IL-6 levels are maintained in a persistent, chronically elevated state in obesity. The effect of chronically elevated serum IL-6 levels on insulin-dependent insulin receptor signaling and insulin action in vivo has not been thoroughly studied, however. Therefore, in the current study, we have examined the effects of chronic IL-6 exposure on insulin sensitivity in vivo. To mimic the constant elevated IL-6 of obesity, we have used implantable osmotic pumps that deliver IL-6 continuously to mice over 5–7 days. We have examined the impact of this chronic cytokine exposure on early insulin-receptor signaling in the liver and muscle and on downstream insulin action in the liver.

RESEARCH DESIGN AND METHODS

Antibodies and reagents. Anti-IRβ (C-19) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti–insulin receptor substrate (IRS)-1, anti–IRS-2, and anti-phosphotyrosine (clone 4G10) antibodies were from Upstate Biotechnology (Lake Placid, NY). Anti-phosphoSTAT3 (Tyr705) was purchased from Cell Signaling Technology (Beverly, MA). Recombinant

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From the [1]Department of Pathology and Laboratory Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York; the [2]Graduate Program in Biochemistry, University of Rochester School of Medicine and Dentistry, Rochester, New York; and the [3]Department of Surgery, University of Rochester School of Medicine and Dentistry, Rochester, New York.

Address correspondence and reprint requests to Robert A. Mooney, Department of Pathology and Laboratory Medicine, University of Rochester School of Medicine and Dentistry, 601 Elmwood Ave., Rochester, NY 14642. E-mail: robert_mooney@urmc.rochester.edu.

Received for publication 25 June 2003 and accepted in revised form 15 August 2003.

CRP, C-reactive protein; IL, interleukin; IRS, insulin receptor substrate; SOCS-3, suppressor of cytokine signaling-3; TNF-α, tumor necrosis factor-α.

human IL-6 was purchased from RDI systems (Flanders, NJ). All other chemicals were from Sigma except where indicated differently.

Animal use. Male C57BL/6 mice purchased from the Jackson Laboratory (Bar Harbor, ME) were used in all studies. The age of animals used in the study ranged from 8–14 weeks for the chronic infusion pump experiments. All animal procedures were in accordance with University of Rochester animal care guidelines and approved by the animal care and use committee.

Chronic IL-6 treatments. For all experiments examining chronic IL-6 exposure, Alzet osmotic pumps (model no. 2001; Durect, Cupertino, CA) with a 7-day pumping capacity and an infusion rate of 1 µl/h were used. Pumps were filled to capacity with 16 µg/ml hIL-6 diluted in carrier (0.9% NaCl and 0.1% BSA). Following induction of halothane general anesthesia, pumps were implanted into the intrascapular subcutaneous space. Incisions were closed with interrupted absorbable sutures.

Insulin treatment and tissue recovery. Two models were used for acute insulin delivery, and both were performed under halothane general anesthesia. For portal insulin treatment the abdomen was opened through a midline incision and the portal vein identified. Insulin (at 300 ng) or carrier (0.9% NaCl and 0.1% BSA) was injected into the portal vein using a 28-gauge needle. Following injection, direct pressure was applied to the portal vein to prevent bleeding. Animals were killed 45 s after portal vein injection. Samples were placed in liquid nitrogen within 15 s. For liver/muscle comparisons a systemic insulin injection was used. The left femoral vein was identified by sharp dissection in the left inguinal region, and the same quantity of insulin was injected intravenously using a 28-gauge needle. Hemorrhage was again prevented with direct pressure from a cotton swab. Tissues were harvested at 90 s after femoral vein injection and frozen in liquid N_2 within 15 s.

Homogenization and preparation of extracts. Frozen liver and muscle were homogenized in 16 volumes (weight/volume) (liver) or 10 volumes (muscle) of lysis buffer (100 mmol/l HEPES pH 7.5, 150 mmol/l NaCl, 1% triton-X-100, 100 mmol/l NaF, 2 mmol/l EDTA, 2 mmol/l EGTA, 10% glycerol, 1 mmol/l benzamidine, 1 mmol/l tetrasodium pyrophosphate, 1 mmol/l PMSF, 5 mmol/l pervanadate, and 1× protease inhibitor cocktail I [Calbiochem]). Frozen tissues were homogenized using the Brinkman PT 10/35 Polytron. Extracts were kept ice-cold at all times. Extracts were cleared by microcentrifugation at 15,000*g* for 10 min at 4°C.

Immunoprecipitation and immunoblotting. Protein content of extracts was determined by the Bradford method (14). Total extract protein (4 mg) was used for all immunoprecipitations. Extracts and immunoprecipitates were resolved by SDS-PAGE and transferred to nitrocellulose. Proteins were detected by immunoblotting and visualized using enhanced chemiluminescence (Amersham-Pharmacia).

Insulin tolerance test. Mice were fasted for 3 h before test. Mice were anesthetized under halothane and insulin (Novagen, Madison, WI) diluted in carrier (0.9% NaCl, 0.1% BSA) was injected intraperitoneally at 0.7 units/kg. Blood glucose levels were determined at indicated intervals using an Accu-Chek (Roche Indianapolis, IN) glucose meter and test strips on tail bleeds of awake mice.

RNA extraction and Northern analysis of glucokinase mRNA. Animals were fasted overnight (16 h). In the morning, a subset of fasted mice were given standard diet and allowed to feed freely for 90 min. Mice were anesthetized, subjected to tail bleeds for glucose levels, and immediately killed for removal of the liver. Total RNA was extracted from the livers of both fasted and refed animals using Trizol (Invitrogen, Carlsbad, CA) extraction. Total RNA (20 µg) was run on a 1% agarose gel containing 1× MOPS buffer and 0.66 mol/l formaldehyde. RNA was transferred to a Zeta-Probe (BioRad, Hercules, CA) membrane overnight, then cross-linked using a Stratalinker (Stratagene, La Jolla, CA). The glucokinase cDNA was a generous gift from Jacob E. Freidman (University of Colorado Health Sciences Center). Hybridization was done at 68°C using Express-Hyb hybrization buffer (Clontech, Palo Alto, CA). For quantitation, the membranes were exposed to a phosphoimager cassette (Molecular Dynamics, Sunnyvale, CA) and scanned in a Storm 840 phosphoimager (Molecular Dynamics). Quantitation was performed using ImageQuant software (Molecular Dynamics). Blots were stripped in boiling 2× SSC buffer with 0.1% SDS until no counts remained. Normalization was done following similar quantitation using a probe to GAPDH.

Analysis of serum IL-6 levels. Following a 5-day constant infusion of hIL-6 at a rate of 16 ng/h, animals underwent general anesthesia and plasma was collected by cardiac puncture with a 25-gauge needle. Human IL-6 and mouse IL-6 Quantikine enzyme-linked immunoassays (R&D Systems, Minneapolis, MN) were used to measure the level of exogenous hIL-6 and endogenous mouse IL-6, respectively. As stated by the manufacturer, there is no cross-reactivity between human and mouse IL-6 in their respective assays. Circulating recombinant hIL-6 is reported as the mean ± SE from 20 animals in 5 experiments. Mouse endogenous IL-6 is reported as the mean ± SD from six mice in two experiments.

Statistics. Densitometric scanning was performed on a Gel Doc gel documentation system (BioRad). Data were analyzed using Quantity One Software (BioRad). Statistical analysis was performed using the Student's *t* test and GraphPad Intuitive Software (GraphPad, San Diego, CA).

RESULTS

Chronic exposure to IL-6 inhibits hepatic insulin receptor signaling. We have previously reported that an acute injection of IL-6 in mice impairs hepatic insulin receptor signaling in response to portal vein injection of insulin. Chronic rather than acute cytokine elevations, however, are characteristic of insulin-resistant states of infection, cachexia, cancer, obesity, and type 2 diabetes (5). We therefore tested the effect of chronic exposure to IL-6 delivered continuously for 5 days using an osmotic pump. The concentration of human recombinant IL-6 in the pumps was such that IL-6 was delivered at 16 ng/h. This dose resulted in an approximate sixfold serum elevation of exogenous hIL-6 over endogenous levels (rhIL-6 112 ± 27 pg/ml and endogenous mouse IL-6 18 ± 5 pg/ml). This elevation approximates that seen in obese individuals, though it does not take into account any differences in species-dependent bioactivity between human and mouse IL-6.

On the 4th day of IL-6 infusion, an overnight fast was initiated. On the 5th day, insulin (300 ng) was administered by intraportal injection and livers were harvested 45 s later. Hepatic insulin receptor autophosphorylation and IRS-1 and -2 tyrosine phosphorylation were analyzed by immunoprecipitation and immunoblot analysis. As expected, insulin stimulated tyrosine phosphorylation of these proteins while IL-6 alone did not. In mice chronically treated with IL-6, however, insulin-dependent insulin receptor autophosphorylation was suppressed by ∼60% (Fig. 1). No consistent change in insulin receptor mass was observed. IL-6 also inhibited insulin-induced IRS-1 tyrosine phosphorylation by ∼60% (Fig. 2*A*) and IRS-2 tyrosine phosphorylation by ∼40% (Fig. 2*B*). Again, mass levels of IRS-1 and -2 were not affected by chronic IL-6 treatment. STAT3 phosphorylation at tyrosine 705 was modestly elevated on day 5 in the liver of mice treated with chronic IL-6 infusion (Fig. 3, compare *lanes 1–4* with *lanes 5–8*). These results demonstrate that chronic IL-6 exposure can suppress early hepatic insulin signaling in mice.

Effect of IL-6 on liver versus skeletal muscle. Approximately 75% of insulin-dependent glucose uptake occurs in the skeletal muscle (15). Since our data indicated that hepatic insulin signaling was reduced in mice chronically treated with IL-6, we sought to understand the insulin sensitivity of muscle compared with that of the liver under these conditions. To this end, mice were exposed to IL-6 for 5 days as described above. Insulin (300 ng) was delivered by femoral vein injection and liver and muscle harvested 90 s later. Insulin receptor signaling in the liver and muscle was then assayed by immunoprecipitation and immunoblot analysis. Again, insulin-dependent tyrosine phosphorylation of insulin receptor, IRS-1, and IRS-2 (Fig. 4, *left panels*) was inhibited in livers of animals treated with chronic IL-6. Thus, for liver insulin receptor signaling, femoral vein injection of insulin recapitulates the results obtained via portal vein injection. However, chronic IL-6 treatment did not affect insulin-dependent insulin receptor

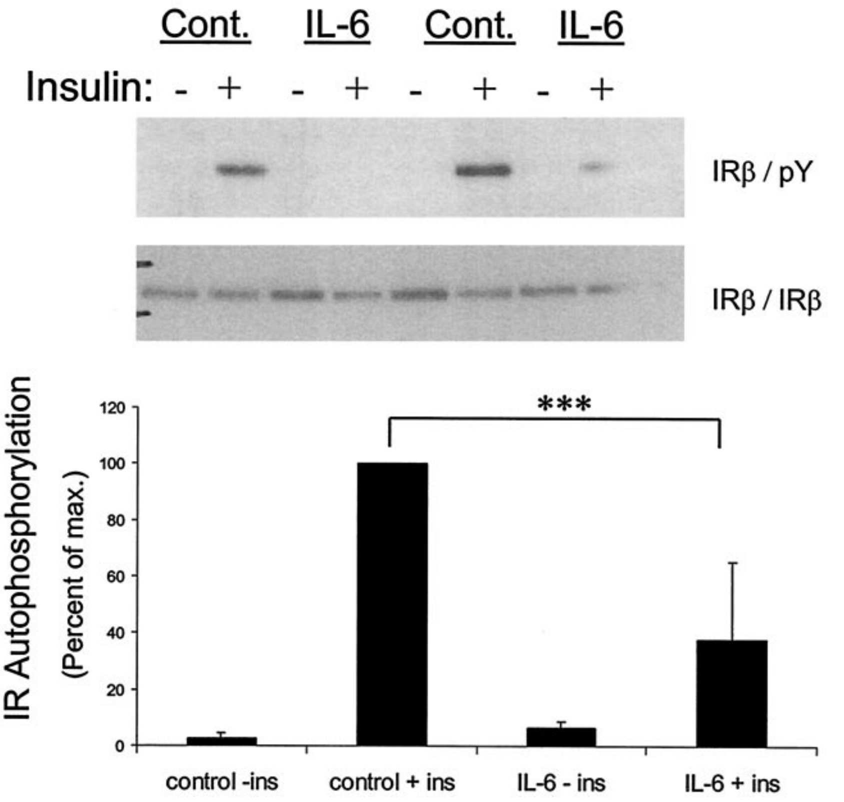

FIG. 1. Chronic exposure to IL-6 suppresses insulin-dependent insulin receptor (IR) autophosphorylation. IL-6 (16 ng/h) or saline was infused using a subcutaneously implanted Alzet osmotic pump. At 5 days, mice were fasted for 16 h before portal vein injection of 300 ng insulin. Immunoprecipitation and Western blot analysis was performed to assess insulin receptor β-subunit (IRβ) tyrosine phosphorylation (pY) and insulin receptor mass. Data represent the mean ± SD of three experiments expressed as percentage of carrier-infused mice treated with insulin. A representative autoradiograph is also shown. ***$P < 0.001$. Immunoprecipitating antibody/blotting antibody are indicated as in IRβ/pY.

signal transduction in the skeletal muscle of these animals (Fig. 4, *right panels*).

In vivo effect of chronic IL-6 treatment on insulin action. To determine the effect of chronic IL-6 exposure on insulin-dependent glucose homeostasis, insulin tolerance tests were performed. Insulin (0.7 units/kg body wt) was administered by intraperitoneal injection, and glucose levels were taken by tail vein sampling at indicated time points (Fig. 5). At early times after insulin injection (t <60 min), no statistical difference in glucose levels between chronic IL-6–treated mice and controls was observed, though glucose levels in IL-6–treated animals trended toward a smaller decrease in response to insulin. After 60 min, however, mice that were chronically treated with IL-6 had significantly higher glucose levels consistent with a modest degree of insulin resistance. A more profound insulin resistance would not be expected given that ~75% of the drop in blood glucose following insulin challenge is due to skeletal muscle glucose uptake, and the inhibitory effect of IL-6 appears to be restricted to the liver, as suggested by the absence of impairment in insulin receptor signaling in skeletal muscle after chronic exposure to IL-6.

The results of the insulin tolerance test suggested that chronic IL-6 exposure altered glucose homeostasis and produced a mild state of insulin resistance in mice. If the liver was the major contributor to this insulin resistance state, it would be anticipated that induction of insulin responsive genes that are central to glucose homeostasis would be compromised. Therefore, we tested whether feeding-dependent glucokinase induction was altered by chronic IL-6 treatment. Maximal glucokinase induction has been shown to occur in mice ~90 min after feeding (16). Mice chronically treated for 5 days with IL-6 (16 ng/h) were fasted overnight and then freely fed for 90 min. Postprandial glucose levels were 24 ± 17% ($n = 6$, $P < 0.05$) higher in the mice infused with 16 ng/h IL-6 relative to controls. Liver glucokinase RNA levels were measured by Northern blot analysis of total RNA. As expected, a large postprandial glucokinase gene induction was observed. This induction was suppressed by ~40% in mice chronically treated for 5 days with 16 ng/h (Fig. 6). These results indicate that chronic IL-6 exposure induces insulin resistance that is manifest on key physiological end points of glucose homeostasis in the liver.

DISCUSSION

The current results provide strong direct evidence that a chronic elevation of circulating IL-6, approximating that observed in obesity and type 2 diabetes, inhibits early hepatic insulin receptor signaling and downstream insulin action in vivo. This provides support for the hypothesis that this proinflammatory cytokine impairs insulin sensitivity and glucose regulation in humans and is linked to obesity-related insulin resistance. These data are particularly consistent with the strong clinical link that has been established between IL-6 and glucose levels (7), obesity, and insulin resistance (3,6,7). We have observed that chronic IL-6 exposure in vivo inhibits the ability of insulin to signal through its receptor and to phosphorylate two major metabolic substrates, IRS-1 and -2. This is consistent with data demonstrating impaired insulin receptor autophosphorylation and substrate phosphorylation in obese and diabetic subjects (17–21). Furthermore, we demonstrated defects in downstream actions of insulin in mice chronically treated with IL-6. These include an abnormal insulin tolerance test, increased postprandial serum glu-

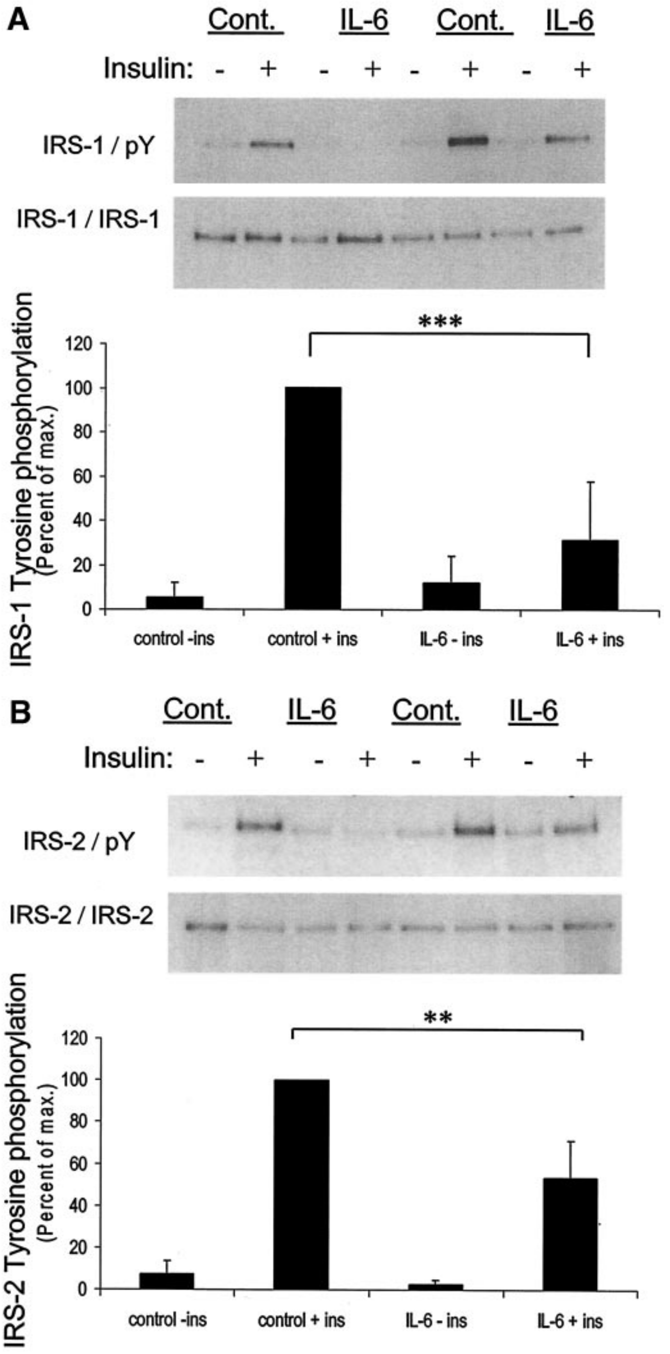

FIG. 2. Chronic exposure to IL-6 suppresses insulin-dependent tyrosine phosphorylation of IRS-1 and -2. Infusion of IL-6, portal vein injection of insulin, and harvesting of liver were performed as described in Fig. 1. Immunoprecipitation and Western blot analysis was performed to assess IRS-1 (*A*) and IRS-2 (*B*) tyrosine phosphorylation and mass. Data represent the mean ± SD of 3–4 experiments expressed as percentage of carrier-infused mice treated with insulin. Representative autoradiographs are also shown. ***$P < 0.001$, **$P < 0.01$. Immunoprecipitating antibody/blotting antibody are indicated as in IRS-1/pY.

cose levels, and reduced postprandial induction of glucokinase. Together, these data demonstrate that chronic exposure to circulating levels of IL-6 that are within the range attainable in obesity and type 2 diabetes are able to recapitulate several of the characteristic metabolic defects of obesity-mediated insulin resistance and diabetes.

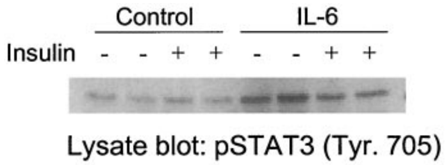

FIG. 3. STAT3 is chronically activated by a 5-day infusion of IL-6. Infusion of IL-6, portal vein injection of insulin, and harvesting of liver were performed as described in Fig. 2. Western blot analysis was performed to assess STAT3 activation in liver homogenates using an anti-Tyr705 STAT3 antibody.

While the current results support a role for IL-6 in mediating hepatic insulin resistance, several additional adipose tissue–derived factors and cytokines have been similarly linked to glucose dysregulation and diabetes. It is likely that multiple cytokines and other factors work in concert with IL-6 to induce obesity-related insulin resistance. This is suggested by the absence of an effect of IL-6 on skeletal muscle. There has been a particular interest in the role of TNF-α in obesity-related insulin resistance. TNF-α has been shown to impair insulin action in vivo and in vitro (22–24). In rodent studies TNF-α overproduction correlates with insulin resistance, and neutralization of TNF-α restored insulin sensitivity to obese rats (25,26). A role for TNF-α is more controversial in humans. TNF-α expression by adipose tissue, but not plasma levels, has been correlated with obesity and insulin resistance (3,27,28). Neutralization of TNF-α in humans did not fully restore insulin sensitivity (29,30). Additionally, a direct role for TNF-α in suppressing insulin receptor signal transduction in the liver has been questioned (25,31,32). Kern et al. (3) reported that IL-6 plasma levels correlate better to insulin resistance than TNF-α levels. However, IL-6 plasma levels in obese subjects are elevated three- to fivefold in these studies (3,6,33). While the role of circulating TNF-α is uncertain, it is possible that an autocrine or paracrine role for TNF-α may exist since TNF-α has been

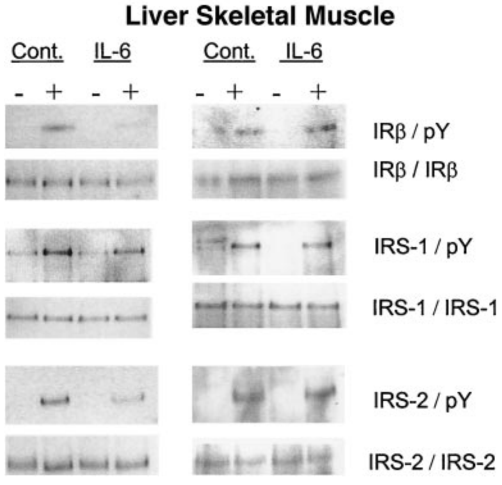

FIG. 4. Suppression of insulin receptor (IR) signaling in response to chronic IL-6 is observed in liver but not skeletal muscle. IL-6 (16 ng/h) or carrier was infused using a subcutaneously implanted Alzet osmotic pump. At 5 days, mice were fasted for 16 h before femoral vein injection of 300 ng insulin. At 1.5 min after injection, liver (*left column*) and quadriceps muscles (*right column*) were harvested and quick-frozen in liquid nitrogen. Insulin-dependent tyrosine phosphorylation of insulin receptor, IRS-1, and IRS-2 were analyzed as in Figs. 2 and 3. Data are representative of three independent experiments. Immunoprecipitating antibody/blotting antibody are indicated as in IRβ/pY.

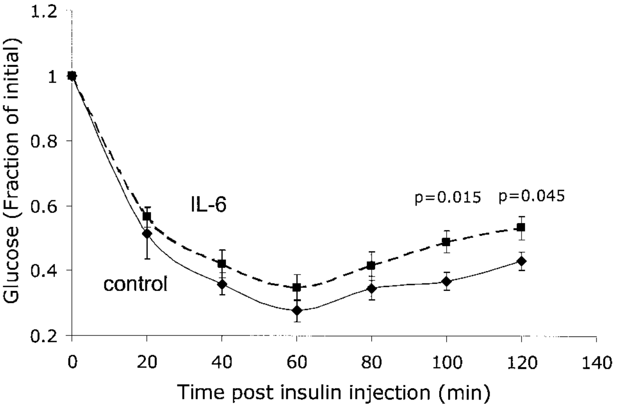

FIG. 5. Modestly impaired insulin response in IL-6–treated mice. Mice were infused for 5 days with IL-6 (16 ng/h) or carrier. Mice were then fasted for 3 h before an insulin tolerance test. Insulin (Novalin; Novagen) was injected intraperitoneally at 0.7 units/kg. Glucose was measured by tail vein sampling at indicated times. Plotted time points represent mean ± SE. Statistically significant differences are indicated with *P* values. Starting blood glucose levels were statistically not different between IL-6– and carrier-treated mice.

shown to induce IL-6 production (30,34,35). It is therefore possible that TNF-α has both a direct effect on insulin responsive tissues, particularly skeletal muscle and adipose tissue, and an indirect effect through induction of IL-6 secretion.

The current in vivo study confirms and extends our finding that IL-6 causes insulin resistance in hepatocytes. Treating HepG2 hepatoma cells and primary mouse hepatocytes with IL-6–inhibited insulin signaling at the level of insulin-dependent IRS-1 tyrosine phosphorylation, phosphatidylinositol 3-kinase association with IRS-1, and AKT/protein kinase B activation (10). Furthermore, insulin-dependent glycogen synthesis was markedly impaired in primary hepatocytes pretreated with IL-6 (10). In the current study, acute and chronic in vivo IL-6 exposure inhibited hepatic insulin sensitivity. Interestingly, chronic exposure to IL-6 for 5 days did not inhibit insulin receptor signaling in skeletal muscle. These results suggest that IL-6 may not play a role in mediating insulin resistance in skeletal muscle. This agrees with reports indicating exceedingly low levels of IL-6 receptors expressed in muscle cells (36,37). Alternatively, the concentrations of IL-6 used in our current study may be too low to elicit a response in skeletal muscle. Several reports have described responses to IL-6 in various models of muscle atrophy, but the IL-6 levels were considerably greater than those observed in obesity and type 2 diabetes, and its effects may not have been direct (38,39). Further investigations are required to clarify the physiological role of skeletal muscle as a target for IL-6 in obesity-mediated insulin resistance.

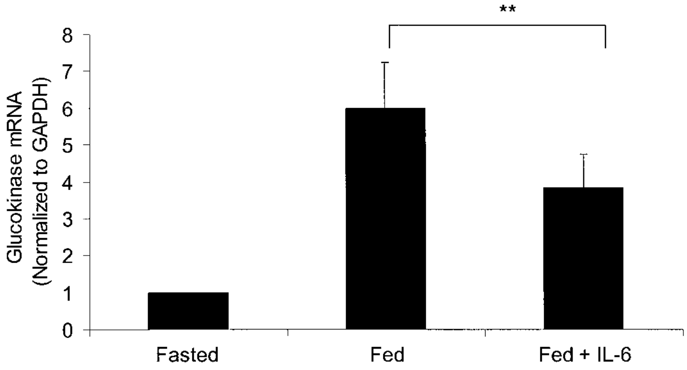

FIG. 6. Chronic IL-6 exposure suppresses postprandial glucokinase induction. Mice at 10 weeks of age were infused for 5 days with carrier (0.9% saline, 0.1% BSA) or IL-6. On the 4th day mice were fasted overnight. Mice were then allowed to freely feed for 90 min, except where indicated, before livers were harvested. Liver RNA was extracted, and 20 μg was used for Northern blot analysis. Bands were quantitated using the Storm 840 phosphoimager and ImageQuant software. Data plotted are the mean ± SD of six determinations performed in two independent experiments. **$P < 0.01$.

How do the proinflammatory cytokines mediate their effects on insulin signaling? TNF-α appears to exert its inhibitory effect by activating several serine kinases, resulting in serine phosphorylation of the IRS-1 and -2 in muscle and adipose tissue (25,40,41). The serine phosphorylated IRS is a poor substrate for the insulin receptor kinase and is also subject to increased degradation (42–44). Although the mechanism through which IL-6 exerts its effect has not been proven in vivo, it is likely that IL-6 acts through a mechanism that is different from that for TNF-α. We have not detected significant serine phosphorylation of IRS-1 in either hepatocytes or liver in response to IL-6 (Klover and Mooney, unpublished observations). Changes in IRS-1 and -2 degradation are also not observed in response to chronic IL-6 treatments (Fig. 2). Our group has strong evidence to suggest that induction of suppressor of cytokine signaling-3 (SOCS-3) by IL-6 in hepatocytes may play a role in mediating the inhibitory properties of IL-6 on the insulin receptor (13). Overexpressed SOCS-3 has been shown to impair insulin-dependent insulin receptor autophosphorylation, IRS-1 tyrosine phosphorylation, phosphatidylinositol 3-kinase association with IRS-1 and AKT/protein kinase B activation in hepatocytes. Furthermore, the timing of SOCS-3 induction by IL-6 correlates with IL-6–induced impairments of insulin receptor signaling in these cells. SOCS-3 is also induced in response to acute IL-6 treatment in the liver of mice (13). Whether SOCS-3 is responsible for the effects of acute or chronic IL-6 exposure in mice is the subject of current investigation.

Changes in the hormonal milieu may be critically important for the development of insulin resistance and type 2 diabetes. The proinflammatory cytokines that are implicated in this disorder may each utilize a distinct mechanism to contribute to the insulin resistance of obesity. The results of this study and our previous report demonstrate that acute as well as chronic exposure to IL-6 inhibits insulin action in vivo. This implicates IL-6 as a contributor to the insulin resistance of obesity, particularly in the liver. Future studies are required to elucidate the mechanism by which IL-6 acts.

ACKNOWLEDGMENTS

This work was supported by U.S. Public Health Service Grants R01-DK38138 and R01-DK60732 to R.A.M.

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