Original Article

Interleukin-6 Increases Insulin-Stimulated Glucose Disposal in Humans and Glucose Uptake and Fatty Acid Oxidation In Vitro via AMP-Activated Protein Kinase

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Although interleukin-6 (IL-6) has been associated with insulin resistance, little is known regarding the effects of IL-6 on insulin sensitivity in humans in vivo. Here, we show that IL-6 infusion increases glucose disposal without affecting the complete suppression of endogenous glucose production during a hyperinsulinemic-euglycemic clamp in healthy humans. Because skeletal muscle accounts for most of the insulin-stimulated glucose disposal in vivo, we examined the mechanism(s) by which IL-6 may affect muscle metabolism using L6 myotubes. IL-6 treatment increased fatty acid oxidation, basal and insulin-stimulated glucose uptake, and translocation of GLUT4 to the plasma membrane. Furthermore, IL-6 rapidly and markedly increased AMP-activated protein kinase (AMPK). To determine whether the activation of AMPK mediated cellular metabolic events, we conducted experiments using L6 myotubes infected with dominant-negative AMPK α-subunit. The effects described above were abrogated in AMPK dominant-negative–infected cells. Our results demonstrate that acute IL-6 treatment enhances insulin-stimulated glucose disposal in humans in vivo, while the effects of IL-6 on glucose and fatty acid metabolism in vitro appear to be mediated by AMPK. *Diabetes* 55:2688–2697, 2006

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ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; DG, deoxyglucose; EGP, endogenous glucose production; FFA, free fatty acid; GIR, glucose infusion rate; IL-6, interleukin-6; IRS, insulin receptor substrate; PI3-K, phosphatidylinositol 3-kinase; rhIL-6, recombinant human IL-6; rmIL-6, recombinant mouse IL-6; SOCS, suppressor of cytokine signaling; TNF-α, tumor necrosis factor-α.

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The pathogenesis of type 2 diabetes is not fully understood, but growing evidence links this disease to a state of chronic inflammation (1), which occurs in tissue such as liver, adipose, and skeletal muscle and results in the secretion of inflammatory cytokines such as resistin, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) from macrophages and/or adipocytes (2). It is thought that elevations in the plasma and/or tissue concentrations of these cytokines have a negative effect on metabolism (3). The role of IL-6 in the etiology of insulin resistance is, however, not fully understood. In mice in vivo, IL-6 has a negative effect on hepatic insulin sensitivity (4), but in healthy humans (5–7) or patients with type 2 diabetes (6), splanchnic glucose output does not increase with acute infusion of recombinant human IL-6 (rhIL-6), while glucose disposal is not impaired (6,7). In our recent study (6), we found that IL-6 did not increase whole-body glucose disposal in either healthy subjects or patients with type 2 diabetes, whereas it reduced insulin concentrations in the patients to values comparable with those of the healthy subjects, suggesting that IL-6 might have favorable effects on insulin action. No studies have tested the effect of IL-6 infusion on insulin action in humans in vivo, and this is the first aim of the present study. We hypothesized that acute IL-6 infusion would enhance insulin-stimulated glucose uptake in humans.

The role of IL-6 in skeletal muscle and adipose tissue in vitro is not clear. IL-6 enhances insulin-stimulated glucose transport (8) or glycogen synthesis (9,10) in myotubes and/or adipocytes, but others (11) have found opposite effects; thus, further studies are warranted. In addition, several studies report that IL-6 increases intramyocellular (6,12) or whole-body (6,13) fatty acid oxidation, which is likely to decrease intramyocellular fatty acid accumulation and can impair insulin signaling (14). Of note, in a recent study, IL-6 was shown to enhance AMP-activated protein kinase (AMPK) in both skeletal muscle and adipose tissue (15). AMPK plays a central role in the regulation of fuel metabolism in skeletal muscle because its activation stimulates fatty acid oxidation (16). Moreover, AMPK may increase glucose uptake (17) via mechanisms thought to involve enhanced insulin signaling transduction (18). However, it is not known whether the effects of IL-6 on glucose and lipid metabolism are mediated by activation of AMPK, and this is an additional aim of the current study.

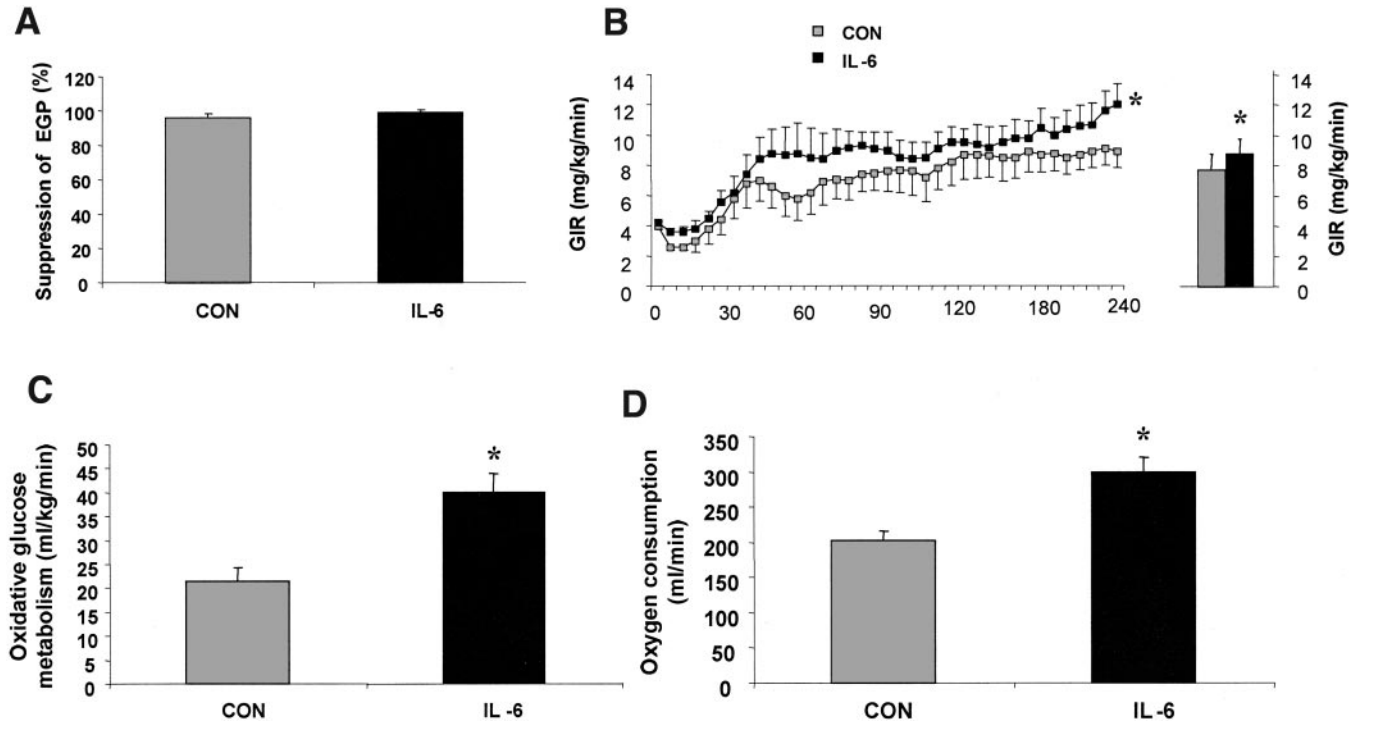

FIG. 1. Suppression of EGP (*A*) and GIR (*B*) during a 240-min hyperinsulinemic-euglycemic clamp in healthy humans and oxidative glucose metabolism (*C*) and whole-body oxygen consumption (*D*) during the final 60 min of the clamp. Subjects were infused with rhIL-6 (IL-6; black symbols) or without (CON; gray symbols) a continuous infusion of recombinant human IL-6 at the rate of 0.5 mg/h. Values are means ± SEM (n = 7). *$P < 0.05$ vs. control.

Accordingly, we performed hyperinsulinemic-euglycemic clamp studies in humans with and without the infusion of rhIL-6 and show that IL-6 enhanced the glucose infusion rate during a clamp. In addition, using cultured L6 myotubes, we show that acute IL-6 treatment increases fatty acid oxidation and glucose uptake in L6 myotubes but not in cells infected with a dominant-negative AMPK α-subunit.

RESEARCH DESIGN AND METHODS

Human experiments. Seven healthy, recreationally active men (aged 24 ± 1 years, weight 80 ± 4 kg, height 183 ± 3 cm) volunteered for this study after having the procedures fully explained, being made aware of all possible risks, and providing written informed consent. The study was approved by the ethical committee of Copenhagen and Frederiksberg communities and performed according to the Declaration of Helsinki.

Experimental protocol. Subjects reported to the laboratory at 0800 after an overnight fast on two occasions separated by 5–7 days and underwent a hyperinsulinemic-euglycemic clamp as previously reported (19) but with the addition of a fatty acid stable isotope infusion. Briefly, at the commencement of the [6,6-$^2H_2$]glucose tracer continuous infusion, a constant infusion of [U-^{13}C]palmitate (0.015 mmol · kg^{-1} · min^{-1}) was also commenced. After the 2-h tracer equilibration period, the hyperinsulinemic-euglycemic clamp commenced and was maintained for 4 h (19). For the duration of the clamp, subjects were also infused with either rhIL-6 or sham (control) in a random, repeated-measures, single-blinded fashion. Our pilot work and data from previous studies (9,10,19) demonstrated that IL-6 delivered in 2% human albumin at a rate of 5 μg/h would predict a plasma concentration of 100–140 pg/ml, a level seen with strenuous exercise.

Blood samples were obtained every 5 min for the first 120 min of the clamp and every 10 min thereafter for the measurement of glucose via the glucose oxidase method (ABL 700; Radiometer, Copenhagen, Denmark). In addition, at 60-min intervals during the clamps, blood samples were collected for the measurement of IL-6, TNF-α, glucose, lactate, free fatty acids (FFAs), glycerol, 6,6 $^2H_2$ glucose enrichments, palmitate and [U-^{13}C]palmitate enrichments, insulin, glucagon, epinephrine, and norepinephrine as previously described (6,19). Breath samples were obtained immediately before tracer infusion and the commencement of the clamp and at 30-min intervals during the clamp for the measurement of oxygen consumption, carbon dioxide production, and ^{13}C breath enrichment (20). Whole-body rates of glucose and fat oxidation were determined assuming a non–protein respiratory quotient using standard equations (21). Glucose and palmitate rates of appearance (R_a) and disappearance (R_d) were determined from changes in percentage enrichment in the plasma of the isotope (20). Because endogenous glucose production (EGP) was totally suppressed in both trials (see RESULTS) and euglycemia was maintained, the glucose infusion rate (GIR) was assumed to be equal to whole-body glucose disappearance. Carbohydrate oxidation rates were then subtracted from the GIR to determine nonoxidative glucose disposal assumed to be representative of glucose storage. Values are presented for the final 60 min of the clamp, since this was the period of steady-state GIR in control subjects (Fig. 1).

In vitro experiments. Cell culture media, serum, and disposables were purchased from Gibco/Invitrogen (Carlsbad, CA). 2-deoxy[U^{14}C]glucose (^{14}C 2-DG) was obtained from Amersham Biosciences (Piscataway, NJ). STAT3, Akt, phospho-Akt, p85 subunit of phosphatidylinositol 3-kinase (PI3-K), acetyl-CoA carboxylase (ACC), and phospho ACC antibodies were purchased from Cell Signaling (Beverly, MA). Insulin receptor substrate (IRS)-1 and phopho-tyrosine clone 4G10 antibodies were obtained from Upstate (Waltham, MA). Horseradish peroxidase–conjugated donkey anti-rabbit secondary antibody was purchased from Amersham; all other antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Cells were treated with human insulin (Actrapid; Novonordisk, Benedikte Østergaard, Denmark) and recombinant mouse IL-6 (rmIL-6) (BenderMed Systems, Burlingame, CA). rmIL-6 was used because it activates both rat and mouse cells, as these species share 87% sequence homology for IL-6 (22).

Cell culture, 2-DG transport, and GLUT4 translocation assay. Cell culture experiments were performed on L6 myotubes and 3T3-L1 adipocytes as previously described (6,23,24). For adenovirus experiments, myotubes were infected with a titerd adenovirus containing control vector (AdGo/GFP) or dominant-negative AMPK mutant (25). For 2-DG, cells were serum-starved for 6 h, after which time they were washed and serum-free media replaced. Glucose uptake measures were then performed using ^{14}C 2-DG as previously described (23). To evaluate the effect of IL-6 on glucose transporter translocation, we utilized L6 myotubes infected with a retrovirus containing an exofacial HA epitope-tagged, human GLUT4 construct (26) and performed GLUT4 translocation assays as described previously (27).

Western blotting. Cells were incubated in serum-free media for 6 h, then IL-6, insulin, and/or wortmannin was added to cells for 10 min, after which they were lysed in ice-cold buffer containing 20 mmol/l HEPES, 2 mmol/l EDTA, 50 mmol/l NaF, 5 mmol/l $Na_4P_2O_7$, and 1% NP40 and phosphatase and

TABLE 1
Plasma hormones and metabolites during the hyperinsulinemic-euglycemic clamp in humans with (rhIL-6) and without (control) IL-6 infusion

	Basal	60 min	120 min	180 min	240 min
Insulin (pmol/l)					
Control	34 ± 5	586 ± 35*	538 ± 26*	546 ± 30*	532 ± 28*
rhIL-6	37 ± 5	561 ± 41*	540 ± 21*	530 ± 17*	512 ± 21*
Glucagon (ng/ml)					
Control	56 ± 8	53 ± 15	54 ± 15	53 ± 13	87 ± 17
rhIL-6	43 ± 8	41 ± 6	37 ± 7	50 ± 8	60 ± 12
Epinephrine (nmol/l)					
Control	0.22 ± 0.03	0.17 ± 0.02	0.19 ± 0.02	0.20 ± 0.04	0.21 ± 0.04
rhIL-6	0.39 ± 0.09	0.32 ± 0.07	0.28 ± 0.08	0.25 ± 0.05	0.31 ± 0.09
Norpinephrine (nmol/l)					
Control	1.05 ± 0.30	1.24 ± 0.23	1.33 ± 0.16	1.18 ± 0.20	1.07 ± 0.27
rhIL-6	0.89 ± 0.13	1.19 ± 0.23	1.08 ± 0.12	0.99 ± 0.08	1.06 ± 0.10
Glucose (mmol/l)					
Control	4.6 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.1
rhIL-6	4.4 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.3 ± 0.1
FFA (μmol/l)					
Control	558 ± 57	195 ± 14*	165 ± 15*	151 ± 15*	151 ± 12*
rhIL-6	522 ± 80	188 ± 18*	171 ± 17*	162 ± 19*	159 ± 17*
Lactate (mmol/l)					
Control	0.7 ± 0.6	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.2	1.1 ± 0.2
rhIL-6	1.0 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1
Glycerol (μmol/l)					
Control	55 ± 7	36 ± 6*	36 ± 6*	28 ± 6*	28 ± 5*
rhIL-6	46 ± 7	38 ± 7*	30 ± 4*	33 ± 8*	30 ± 7*

Data are means ± SE ($n = 7$). *Difference ($P < 0.05$) from basal.

protease inhibitor cocktails. Lysates were then spun at 16,000*g* for 5 min and protein concentrations determined using commercially available reagents (Pierce Biotechnology, Rockford, IL). Western blotting was then performed as described elsewhere (23).

AMPK activity and palmitate oxidation. Lysates were incubated with AMPK-α1 and -α2 antibody-bound protein A agarose beads for 2 h; immunocomplexes were washed and AMPK activity determined in the presence of 200 μmol/l AMP (23,28). Palmitate oxidation experiments were performed as previously described (6,23).

Transgenic mice experiments. Four-month-old male IL-6 knockout mice generated by Kopf et al. (29) and littermate control mice (wild type) were used in these experiments. All procedures were approved by the RMIT Animal Ethics Committee. Mice were maintained on a 12-h light/dark cycle with lights on at 0700 and fed a standard rodent chow diet/low-fat diet (Harlan Teklad, Madison, WI). Mice were anesthetized with pentobarbital sodium (60 mg/kg body wt) and injected i.p. with either 0.7 units/kg insulin or an equal volume of saline. Gastrocnemius muscles were rapidly excised after 5 min and snap frozen in liquid nitrogen. Protein was extracted and subsequently analyzed for insulin signaling by Western blot. In addition, in animals injected with saline, quadriceps were rapidly excised, snap frozen in liquid nitrogen, and analyzed for AMPK and ACC phosphorylation as described above.

Statistical analyses. Data were analyzed using a one- or two-way ANOVA with repeated measures (Statistica, Tulsa, OK), with significance accepted at $P < 0.05$. If analyses revealed a significant interaction, a Newman-Keuls post hoc test was used to locate specific differences. Data are presented as means ± SE.

RESULTS

IL-6 does not affect the suppression of endogenous glucose production but increases GIR and glucose oxidation during a hyperinsulinemic-euglycemic clamp in healthy humans. We first performed 4-h hyperinsulinemic-euglycemic clamps in healthy humans to assess the effect of IL-6 on insulin action. Infusion of rhIL-6 during the clamp increased circulating IL-6 to an average of 195 ± 71 pg/ml, whereas it remained at basal levels (3 ± 1 pg/ml) in the control trial. TNF-α was not increased above basal levels in either the rhIL-6 or control trial, averaging 3.1 ± 0.3 and 2.9 ± 0.3 pg/ml, respectively. During the clamp, plasma insulin increased in both trials, averaging 550 ± 27 and 534 ± 24 pmol/l for the control and rhIL-6 trials, respectively. Plasma glucagon, epinephrine, norepinephrine, glucose, and lactate were neither affected by the clamp procedure nor the rhIL-6 infusion (Table 1). Plasma FFAs and glycerol were not different when comparing rhIL-6 with control at rest or during the clamp, but plasma FFA and glycerol were reduced in both trials during the clamp (Table 1).

In experiments in mice, IL-6 has been shown to inhibit the suppression of EGP by insulin (4). In the present study, EGP was completely suppressed during the hyperinsulinemic-euglycemic clamp with or without the infusion of rhIL-6 (Fig. 1*A*). rhIL-6 infusion resulted in a ~15% increase in GIR (Fig. 1*B*). Although this was modest, it was observed in all subjects ($P < 0.001$). Basal oxygen consumption (V_{O_2}) and carbon dioxide production (V_{CO_2}) were not different when comparing control with rhIL-6, while the basal respiratory exchange ratio averaged 0.80 ± 0.06 and 0.82 ± 0.02 for control and rhIL-6, respectively. During the steady-state period of the clamp, both oxidative glucose metabolism (Fig. 1*C*) and V_{O_2} (Fig. 1*D*) were higher with the infusion of rhIL-6. Nonoxidative glucose metabolism was not affected by IL-6 infusion (data not shown).

IL-6 does not affect whole-body lipolysis or fatty acid oxidation during a hyperinsulinemic-euglycemic clamp in healthy humans. Using a pulse-chase technique in rodents, Bruce and Dyck (12) demonstrated that IL-6 attenuated the suppressive effect of insulin on fatty acid oxidation, increasing exogenous fatty acid oxidation and reducing the esterification of fatty acid to triacylglycerol. We sought to determine whether IL-6 had this effect in humans in vivo. Plasma fatty acid rate of appearance (R_a) and disappearance (R_d) were markedly reduced at the

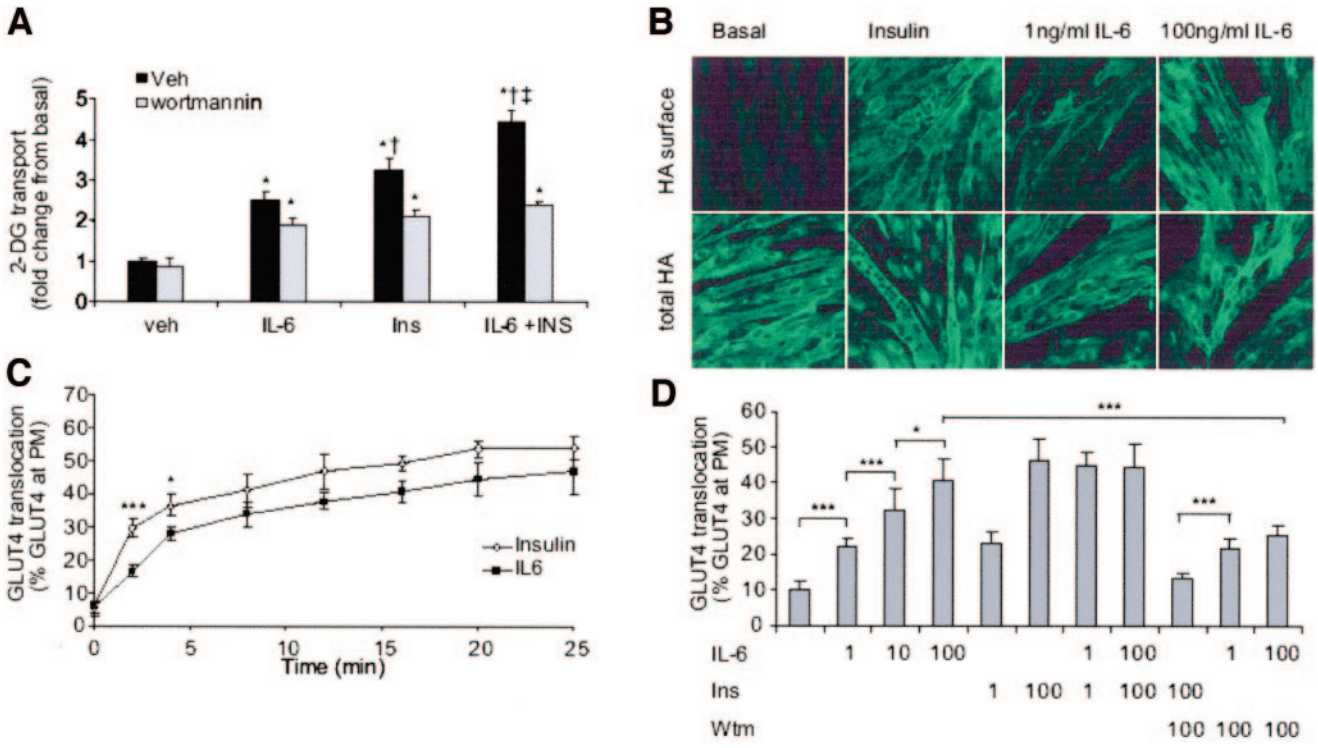

FIG. 2. Rates of ^{14}C 2-DG transport in L6 myotubes treated with rmIL-6 (100 ng/ml, 120 min) and/or insulin (100 nmol/l, 30 min) and/or the PI3-K inhibitor wortmannin (100 nmol/l, 120 min) (*A*). GLUT4 translocation experiments are shown in *B–D*. In *B* and *C*, doses were identical to treatments in *A*. In *D*, the treatment times were identical to treatments in *A*. *A*: *Significantly greater than vehicle treatment, †significantly greater than IL-6 treatment, ‡significantly greater than insulin treatment, $P < 0.05$. *C*: *$P < 0.05$ and ***$P < 0.01$ compared with 0 min. *D*: *$P < 0.05$ and ***$P < 0.01$ as indicated.

onset of the euglycemic-hyperinsulinemic clamp with the fatty acid R_d averaging 0.14 ± 0.07 and 0.07 ± 0.03 mmol · kg^{-1} · min^{-1} for control and rhIL-6, respectively. With such a low fatty acid R_d, and the fact that the respiratory exchange ratio averaged 1.00 ± 0.01 and 1.00 ± 0.05 for control and rhIL-6, respectively, during the clamp, it was not surprising that we were unable to detect measurable ^{13}C enrichments in breath CO_2 samples, consistent with insulin completely suppressing fat oxidation with or without rhIL-6 infusion.

IL-6 increases basal and insulin-stimulated 2-DG uptake and GLUT4 translocation to the plasma membrane. In our human experiment, muscle samples were not obtained. Because skeletal muscle accounts for ~80% of whole-body insulin-stimulated glucose disposal (30), and in an attempt to elucidate a mechanism for our findings in the setting where the effect of IL-6 on glucose metabolism in vitro is controversial (8,11), we sought to examine the effect of IL-6 on basal and insulin-stimulated transport rates of ^{14}C 2-DG in L6 myotubes. We first performed dose-response experiments in L6 myotubes. At a dose of 1 ng/ml, IL-6 increased basal glucose uptake, and while this effect was increased when the dose was increased to 10 ng/ml, no further effect was observed at 100 or 1,000 ng/ml IL-6. In the insulin-stimulated condition, 1 ng/ml was not sufficient to augment glucose uptake, but at concentrations ranging from 10 to 1,000 ng/ml IL-6, insulin-stimulated glucose uptake was increased by IL-6. These experiments were performed at 100 nmol/l insulin because, in preliminary experiments, we did not see an effect at lower doses of insulin. As a consequence of these initial studies, further experiments used 100 ng/ml IL-6 and 100 nmol/l insulin. IL-6 increased both basal and insulin-stimulated glucose transport (Fig. 2*A*). Because IL-6 has been shown by others to increase glycogen synthesis via enhanced insulin signaling transduction (9), we next performed experiments using the PI3-K inhibitor wortmannin. The addition of wortmannin (100 nmol/l) resulted in an inhibition of the IL-6–induced increase in both basal and insulin-stimulated glucose uptake (Fig. 2*A*).

In view of the effects of IL-6 on glucose transport, we next set out to determine the effects of IL-6 on GLUT4 translocation in L6 cells. In L6 myotubes, we observed that maximal doses of insulin increased cell surface levels of GLUT4 by four- to fivefold. At low doses of IL-6 (1 ng), we observed a twofold increase in cell surface levels of GLUT4, and this increased in a dose-response manner (Fig. 2*D*). We observed an additive effect of IL-6 and insulin on GLUT4 translocation at submaximal IL-6 concentrations, suggesting that these agonists may augment this process via distinct mechanisms. The kinetics of IL-6 and insulin-stimulated GLUT4 translocation were similar in L6 myotubes, occurring with a half-time of 2–3 min. Furthermore, whereas the effects of insulin on GLUT4 translocation were completely inhibited by the PI3-K inhibitor wortmannin, this drug had little effect on IL-6–stimulated GLUT4 translocation at low doses of the cytokine (1 ng/ml). Intriguingly, at higher doses of IL-6 (100 ng/ml), we did observe a partial inhibition of IL-6–stimulated GLUT4 translocation by wortmannin.

Effect of IL-6 on muscle cell signal transduction pathways. To establish that IL-6 was signaling through the IL-6 receptor/leukemia inhibitory factor receptor/gp130 receptor complex, we quantified the phosphorylation of one of the prime downstream targets of the receptor complex, STAT3 (Tyr^{705}), and the downstream protein

suppressor of cytokine signaling (SOCS)3. Phosphorylation of STAT3 was increased after only 10 min of treatment with IL-6 (Fig. 3*A*), while SOCS3 protein expression was elevated after 60 min of exposure (Fig. 3*B*). Insulin did not activate STAT3 or affect SOCS3, nor did it affect the IL-6 response seen in Fig. 3*A* and *B* (data not shown).

IL-6 was shown to activate AMPK in one previous study (15). To investigate this further, we treated L6 myotubes with IL-6 for 10–120 min and examined the effect on AMPK signaling. In addition, we treated cells for 30 min with 2 mmol/l 5-amino-4-imidazolecarboxamide riboside (AICAR) (a pharmacological activator of AMPK), insulin, or IL-6 plus insulin for 10 min. IL-6 increased phosphorylation of AMPK (Thr^{172}) and its downstream target ACCβ Ser^{218} within 10 min, an affect that persisted for 120 min (Fig. 3*C* and *D*). We also found that insulin had no effect on AMPK (Thr^{172}) and ACCβ (Ser^{218}) phosphorylation, nor did it affect the response to IL-6 treatment alone, which was comparable to treatment with AICAR (Table 2). We next examined the capacity for IL-6 to phosphorylate AMPK (Thr^{172}) at various IL-6 concentrations (Fig. 3*E*). Consistent with the IL-6 dose-response experiments on glucose uptake and GLUT4 translocation, the threshold for AMPK phosphorylation was 1 ng/ml with maximal response achieved in the range of 10 to 100 ng/ml (Fig. 3*E*).

Given that IL-6 increased glucose uptake and GLUT4 translocation, we next examined the effect of increased IL-6 on insulin signaling processes. We measured the effect of IL-6 on basal and insulin-stimulated tyrosine phosphorylation of IRS-1, the subsequent association of IRS-1 with the p85 catalytic subunit of PI3-K and the phosphorylation of Akt (Ser^{473}). Despite the fact that IL-6 treatment increased glucose uptake and GLUT4 translocation, effects that were sensitive to wortmannin treatment, we found no evidence that IL-6 treatment alone increased insulin signaling processes (Fig. 3*F*–*H*). As expected, insulin increased tyrosine phosphorylation of IRS-1 (Fig. 3*F*), the association of IRS-1 with the p85 catalytic subunit of PI3-K (Fig. 3*G*), and phosphorylation of Akt at Ser^{473}. However, despite the fact that the combination of insulin plus IL-6 increased glucose uptake compared with insulin alone (Fig. 2*A*) and that there was an additive effect of insulin plus low-dose IL-6 on GLUT4 translocation (Fig. 2*D*), we found little evidence that IL-6 increases insulin-stimulated signaling. Neither insulin-stimulated tyrosine phosphorylation of IRS-1 (Fig. 3*F*) nor the association of IRS-1 with the p85 catalytic subunit of PI3-K (Fig. 3*G*) was affected by IL-6 treatment, while there was a small, albeit significant, increase in insulin-stimulated phosphorylation of Akt (Ser^{473}) with IL-6 treatment (Fig. 3*A*).

Effects of IL-6 on muscle signal transduction in vivo. Next, to determine the effects on muscle signal transduction in vivo, we assessed skeletal muscle from IL-6 knockout mice previously found to be obese and glucose intolerant (31). We found AMPK-α2 and ACC phosphorylation was reduced in IL-6 knockout mice, whereas AMPK-α1 activity was not significantly affected, confirming earlier findings (15). Of note, insulin signal transduction was not different when comparing insulin-stimulated muscle from IL-6 knockout mice with littermate control mice (data not shown), indicating that IL-6 is not involved in insulin signal transduction in vivo.

The effects of IL-6 on basal and insulin-stimulated glucose uptake and fatty acid oxidation are abrogated in L6 myotubes infected with an AMPK dominant-negative construct. When examining our signal transduction data, the most marked and rapid effect appeared to be the IL-6–mediated increase in AMPK signaling. To determine whether activation of AMPK was responsible for the observed metabolic events, we tested the effects of IL-6 on glucose uptake L6 myotubes adenovirally infected with an AMPK dominant-negative construct (Fig. 4*A*). We also compared the effect of mock infection (ad null) with uninfected cells on insulin-stimulated glucose uptake and observed no differences (data not shown). AMPK dominant-negative adenovirus treatment inhibited IL-6–induced increases in basal and insulin-stimulated glucose uptake (Fig. 4*A*). Consistent with these data, IL-6 treatment increased ACCβ (Ser^{218}) phosphorylation in ad null but not in ad AMPK dominant-negative cells (Fig. 4*B*). IL-6 is known to increase fat oxidation (6,12,13) and activate AMPK (15). To determine whether the effect of IL-6 on fat oxidation was AMPK mediated, we next performed palmitate oxidation experiments in our AMPK dominant-negative and ad null cells. As expected, IL-6 markedly increased palmitate oxidation in ad null cells, but this effect was eliminated in AMPK dominant-negative cells, demonstrating a dependency on activation of AMPK for the stimulatory effects of IL-6 on fatty acid oxidation (Fig. 4*C*).

The cellular effects of IL-6 seen in L6 myotubes are also seen in 3T3-L1 adipocytes. Adipose tissue is important in the etiology of insulin resistance, and the effect of IL-6 on glucose metabolism in 3T3-L1 adipocytes is unclear (8,11). Accordingly, we next sought to determine whether our results seen in L6 myotubes persisted in 3T3-L1 adipocytes. Consistent with our results observed in L6 myotubes, we found that IL-6 signals through STAT3 and increases SOCS3 (Fig. 5*A* and *B*). We also showed that IL-6 increases basal and insulin-stimulated glucose transport (Fig. 5*C*).

DISCUSSION

It is generally thought that IL-6 promotes insulin resistance (3). Here, we show that acute IL-6 enhances insulin-stimulated glucose disposal in humans in vivo, while the effects of IL-6 on glucose and lipid metabolism in vitro appear to be mediated by AMPK. Our human data demonstrate that acute elevations in IL-6 results in an increase in insulin-stimulated whole-body glucose disposal. It is well known that IL-6 is rapidly released into the circulation following exercise (32). Hence, our data may provide a mechanism for the enhanced insulin action typically observed after a single exercise bout (33).

An important finding from the present study was that rhIL-6 infusion during a hyperinsulinemic-euglycemic clamp in healthy humans did not effect the insulin-mediated suppression of EGP, while increasing GIR. These data are in contrast with the observations reported in mice (4), where IL-6 infusion for 120 min resulted in a marked approximate twofold decrease in GIR during a subsequent 120-min hyperinsulinemic-euglycemic clamp. Hepatic sensitivity to insulin appears to be greater in humans than rodents (34,35), and this fact, taken together with previous results from our group (6), which suggest that IL-6 might enhance insulin-stimulated glucose disposal, provided the rationale for performing the human study. From our data, we conclude that the acute IL-6–induced hepatic insulin resistance observed in rodent studies cannot extend to studies in humans. Therefore, the generally negative ef-

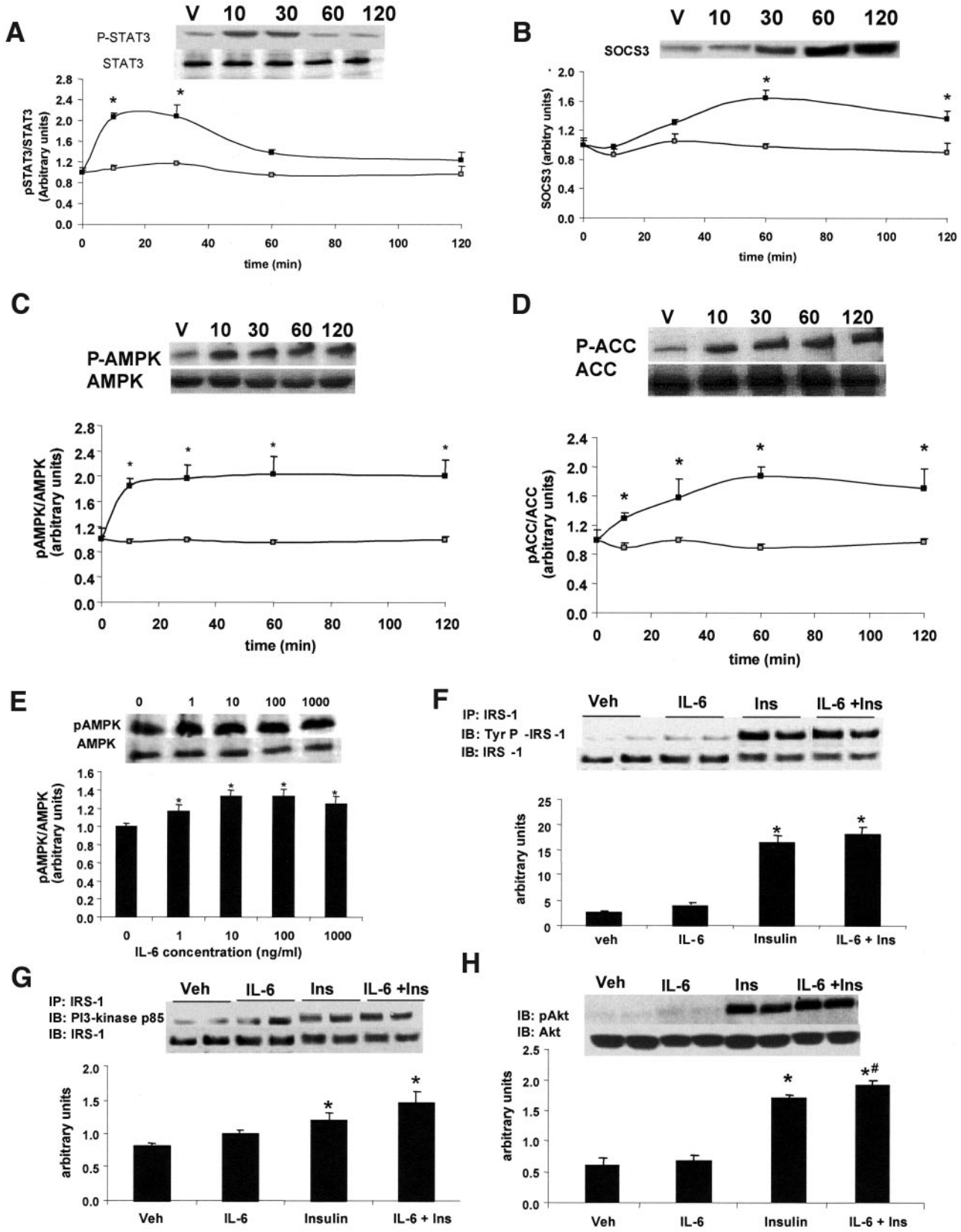

FIG. 3. Phosphorylation of STAT3 (Tyr^{705})/total STAT3 (*A*) and SOCS3 protein abundance (*B*), phosphorylation of AMPK (Thr^{172})/total AMPK (*C*) and ACC (Ser^{218})/total ACC (*D*) in L6 myotubes treated with rmIL-6 (black squares) or vehicle (gray squares). Phosphorylation of AMPK (Thr^{172})/total AMPK in L6 myotubes treated with vehicle or rmIL-6 at doses ranging from 1 to 1,000 ng/ml (*E*). Tyrosine phosphorylation of IRS-1 (*F*), IRS-1 association with the p85 subunit of PI3-K (*G*), and phosphorylation of Akt (Ser^{473})/total Akt protein (*H*) in L6 myotubes are shown. In all experiments except *E*, cells were treated with rmIL-6 (100 ng/ml) and/or insulin (100 nmol/l). *Significantly greater than vehicle treatment and #significantly greater than insulin treatment, $P < 0.05$.

TABLE 2
Effect of IL-6, insulin, and/or AICAR treatment on AMPK and ACC phosphorylation in L6 myotubes

	Vehicle	IL-6	Insulin	IL-6 + insulin	AICAR
pAMPK/AMPK	1.00 ± 0.20	1.83 ± 0.13*	1.29 ± 0.60	1.78 ± 0.12*	1.95 ± 0.20*
pACC/ACC	1.00 ± 0.16	1.29 ± 0.08*	1.01 ± 0.26	1.14 ± 0.18*	1.72 ± 0.38

Data are means ± SE (n = 6). *Difference from Vehicle. AICAR, 5-amino-4-imidazolecarboxamide riboside.

fects of IL-6 observed in murine models in vivo (4,36,37) must be interpreted with a degree of caution.

In the current study, we attempted to infuse a dose of IL-6 that resulted in a circulating concentration of ~120 pg/ml, which has been observed in humans after a marathon run (38). Unfortunately, our concentrations were higher than this. Notwithstanding this, the dose administered (5 μg/h) did not result in clinical symptoms of sepsis, such as elevated temperature and/or shivering, consistent with previous studies (6,7,13), and the subjects were unable to predict if they were being infused with IL-6 or control. Hence, the present data, together with our recent findings on the antidiabetic effect of ciliary neurotrophic factor (23,39,40), an IL-6 family cytokine, suggest that there is merit in future investigations into the efficacy of using gp130 receptor peptides as possible drug therapies to treat insulin resistance.

As a corollary to our human in vivo study, we performed a number of experiments in L6 myotubes to understand the mechanism(s) as to how IL-6 may affect glucose metabolism. From our in vivo experiments, we can conclude that IL-6 increases basal and insulin-stimulated glucose uptake via an increase in translocation of GLUT4 from intracellular pools to the plasma membrane. The effects of IL-6 on glucose metabolism were abolished in AMPK dominant-negative–infected cells. In contrast with other studies (10), we found no evidence that IL-6 increases insulin signaling, even though the PI3-K inhibitor wortmannin appeared to have an effect on IL-6–induced glucose uptake and GLUT4 translocation. Hence, our data suggest, at least in vitro, that AMPK is a mechanism whereby IL-6 exerts its effect on cellular glucose uptake, although we cannot rule out the possibility that other kinases, which share a substrate with AMPK, are also involved.

Recent studies have shown that IL-6 can enhance lipid oxidation in vitro (6), ex vivo (12), and in vivo (6,13). In addition, a recent study has also demonstrated that IL-6 activates AMPK (15). It is well known that AMPK phosphorylates ACC resulting in inhibition of ACC activity,

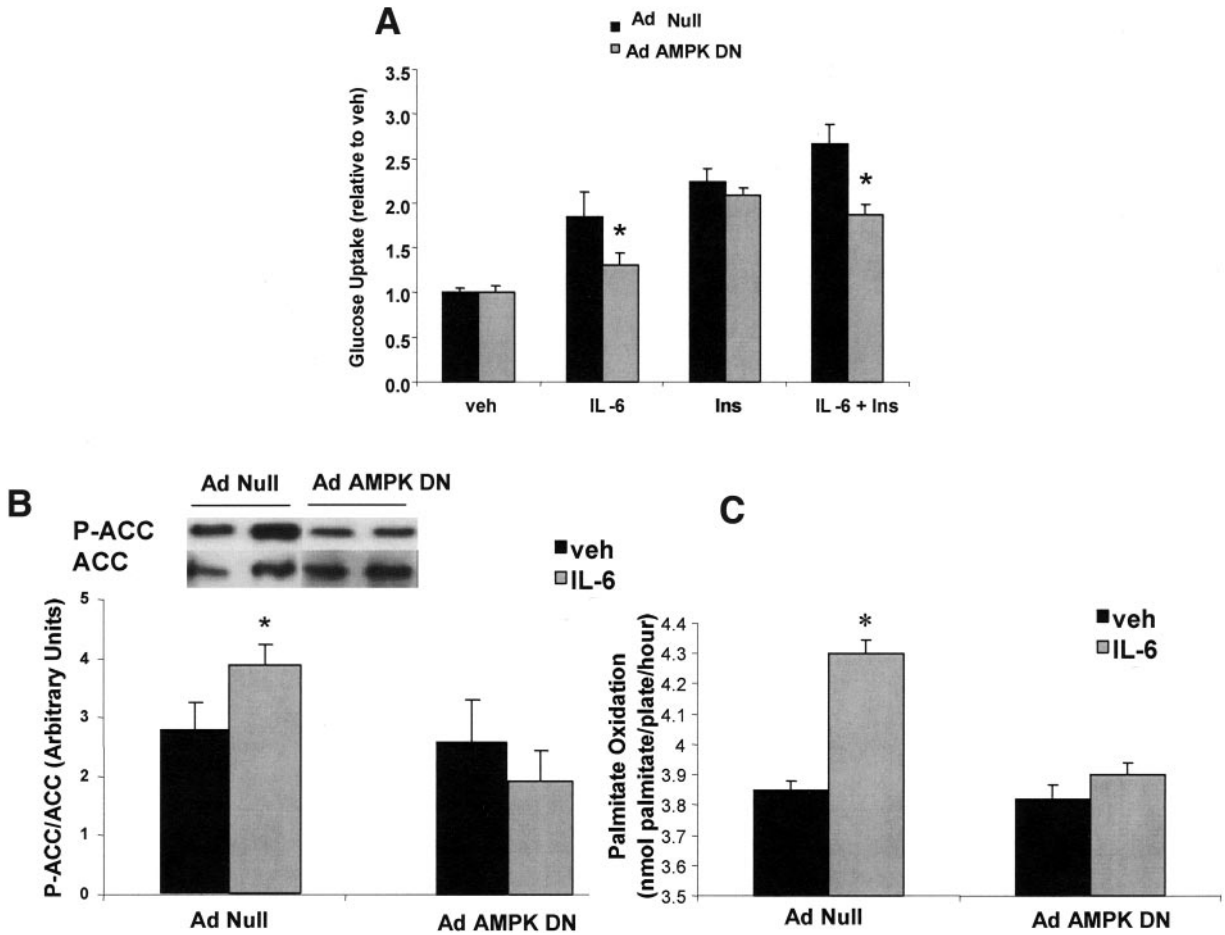

FIG. 4. Rates of ^{14}C 2-DG transport in L6 myotubes (*A*), phosphorylation of ACC (Ser^{218})/total ACC (*B*), and ^{14}C palmitate oxidation (*C*) in L6 myotubes infected with titerd adenovirus containing control vector (ad null) or dominant-negative (DN) AMPK mutant. Cells were treated with rmIL-6 (100 ng/ml) for 120 min and/or insulin (100 nmol/l) for 30 min. *Difference from ad null, $P < 0.05$.

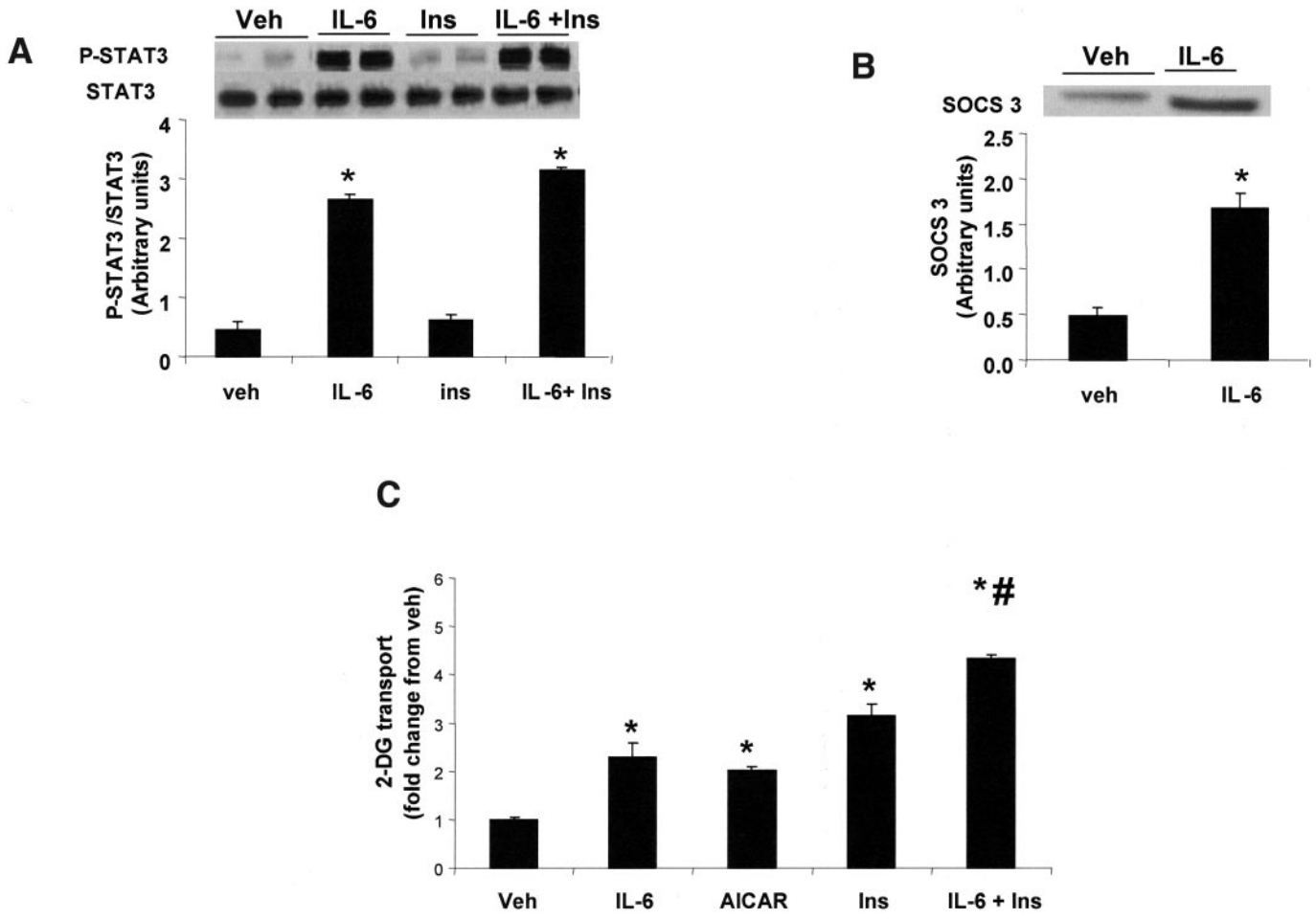

FIG. 5. Phosphorylation of STAT3 (Tyr^{705})/total STAT3 (*A*) and SOCS3 protein abundance (*B*) and rates of ^{14}C 2-DG transport (*C*) in 3T3-L1 adipocytes. Doses and time courses are identical to similar experiments performed in L6 myotubes. *Significantly greater than vehicle treatment and #significantly greater than insulin treatment, $P < 0.05$.

which in turn leads to a decrease in malonyl CoA content, relieving inhibition of CPT-1 and increasing fatty acid oxidation (16). Whether the IL-6–induced increase in lipid oxidation is mediated by the activation of AMPK had not been elucidated, but we show, via the use of an AMPK dominant-negative–infected cell line, that IL-6–mediated phosphorylation of ACC and subsequent palmitate oxidation is AMPK dependent. Our data, together with our recent findings regarding ciliary neurotrophic factor (23), suggest that ligands that bind to the gp130 receptor complex can markedly enhance fat oxidation via activation of AMPK.

IL-6 has been shown to activate SOCS proteins in liver (41), and hepatic overexpression of SOCS1 and -3 in mice leads to liver insulin resistance (42). Paradoxically, in our study, while IL-6 increased SOCS3 expression in myotubes, it also increased glucose uptake in these cells. Given the negative effects of SOCS3 on insulin action, this observation was unexpected. In our study, we examined the effects of acute IL-6 treatment on insulin action, and while we found that that this treatment protocol enhanced insulin action, Rotter et al. (11) found opposite effects when adipocytes were subjected to a 24-h IL-6 treatment protocol. Therefore, we cannot rule out the possibility that prolonged treatment with IL-6 that results in persistent elevations of SOCS3 may have negative effects on insulin action.

Weigert et al. (43) recently compared the relative fold induction of SOCS3 mRNA in skeletal muscle and liver from mice treated with IL-6 in vivo. While IL-6 increased SOCS3 twofold in muscle (comparable with our fold induction in SOCS3 protein expression in vitro), it was increased ~25-fold in liver, suggesting that capacity for IL-6 to induce SOCS3 is much greater in hepatic tissue. Although speculative, the possibility exists that the negative effects of IL-6–induced increases in SOCS3 in tissues such as skeletal muscle may be overridden by the positive effects of activation of AMPK.

While our in vitro experiments provide solid evidence that the activation of AMPK is a major mechanisms by which IL-6 exerts its metabolic effect on glucose and lipid metabolism, we cannot make any definitive statements as to the mechanism by which rhIL-6 enhanced insulin-stimulated glucose disposal in humans in vivo because we were unable to obtain skeletal muscle or adipose tissue biopsies in our human experiment. However, given that the suppressive effect of insulin was not altered by rhIL-6 infusion, an increase in whole-body glucose disposal must account for the increase in GIR. Whether this was due to enhanced uptake of glucose by skeletal muscle, adipose, or both requires further investigation. One major finding of the present study was the observation that rhIL-6 infusion resulted in an increase in glucose oxidation rather than nonoxidative glucose disposal, a finding consistent with

previous studies in rodents (44). As discussed, we did not measure the AMPK activity in the human in vivo experiment. However, the observed increase in glucose oxidation rather than nonoxidative glucose disposal may argue against AMPK playing a role in the enhanced glucose disposal because mice with a mutation in the γ-3 subunit of AMPK that results in chronically activated AMPK have very high muscle glycogen levels, suggesting that AMPK enhances glucose storage rather than oxidation (45). Hence, further research in humans in vivo is warranted.

In summary, our results demonstrate that acute IL-6 treatment increases insulin-stimulated glucose disposal in humans in vivo. In addition, IL-6 increases fatty acid oxidation, glucose transport, and GLUT4 translocation to the plasma membrane in vitro. Activation of AMPK by IL-6 appears to play an important role in modulating some of these metabolic effects. Our data suggest that ligands that activate the heterodimeric IL-6 receptor/gp130β receptor complex in muscle and fat cells are a viable drug target to treat peripheral insulin resistance.

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