Original Article

Interleukin-6 Directly Increases Glucose Metabolism in Resting Human Skeletal Muscle

Stephan Glund,[1] Atul Deshmukh,[1] Yun Chau Long,[1] Theodore Moller,[1] Heikki A. Koistinen,[2] Kenneth Caidahl,[3] Juleen R. Zierath,[1] and Anna Krook[1,4]

Interleukin (IL)-6 is a proinflammatory cytokine shown to modify insulin sensitivity. Elevated plasma levels of IL-6 are observed in insulin-resistant states. Interestingly, plasma IL-6 levels also increase during exercise, with skeletal muscle being the predominant source. Thus, IL-6 has also been suggested to promote insulin-mediated glucose utilization. In this study, we determined the direct effects of IL-6 on glucose transport and signal transduction in human skeletal muscle. Skeletal muscle strips were prepared from vastus lateralis biopsies obtained from 22 healthy men. Muscle strips were incubated with or without IL-6 (120 ng/ml). We found that IL-6 increased glucose transport in human skeletal muscle 1.3-fold ($P < 0.05$). A 30-min pre-exposure to IL-6 did not affect insulin-stimulated glucose transport. IL-6 also increased skeletal muscle glucose incorporation into glycogen, as well as glucose oxidation (1.5- and 1.3-fold, respectively; $P < 0.05$). IL-6 increased phosphorylation of STAT3 (signal transducer and activator of transcription 3; $P < 0.05$), AMP-activated protein kinase ($P = 0.063$), and p38 mitogen-activated protein kinase ($P < 0.05$) and reduced phosphorylation of S6 ribosomal protein ($P < 0.05$). In contrast, phosphorylation of protein kinase B/Akt, AS160 (Akt substrate of 160 kDa), and GSK3α/β (glycogen synthase kinase 3α/β) as well as insulin receptor substrate 1–associated phosphatidylinositol 3-kinase activity remained unaltered. In conclusion, acute IL-6 exposure increases glucose metabolism in resting human skeletal muscle. Insulin-stimulated glucose transport and insulin signaling were unchanged after IL-6 exposure. *Diabetes* 56:1630–1637, 2007

From the [1]Department of Molecular Medicine and Surgery, Section for Integrative Physiology, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; [2]Helsinki University Central Hospital and Biomedicum, Helsinki, Finland; the [3]Department of Molecular Medicine and Surgery, Section for Clinical Physiology, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; and the [4]Department of Physiology and Pharmacology, Section for Integrative Physiology, Karolinska Institutet, Stockholm, Sweden.

Address correspondence and reprint requests to Anna Krook, Department of Physiology and Pharmacology, Section for Integrative Physiology, Karolinska Institutet, von Eulers väg 4, SE-171 77 Stockholm, Sweden. E-mail: anna.krook@ki.se.

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AMPK, AMP-activated protein kinase; AS160, Akt substrate of 160 kDa; GSK, glycogen synthase kinase; IL, interleukin; IRS, insulin receptor substrate; KHBB, Krebs-Henseleit bicarbonate buffer; MAPK, mitogen-activated protein kinase; PKB, protein kinase B; STAT, signal transducer and activator of transcription.

Obesity is associated with an inflammatory state of adipose tissue. Pro-inflammatory cytokines, such as tumor necrosis factor-α and interleukin (IL)-6, have been suggested to contribute to the manifestation of whole-body insulin resistance (1). Although several lines of evidence implicate tumor necrosis factor-α as a contributing factor in the manifestation of peripheral insulin resistance (2), the role of IL-6 has been challenged (3,4).

Adipocytes, monocytes/macrophages, fibroblasts, and vascular endothelial cells are important sources of IL-6 production, with adipocytes secreting up to 35% of total circulating levels (5,6). In type 2 diabetic and nondiabetic obese humans, adipose tissue IL-6 content correlates with impaired insulin-mediated glucose uptake (5,7). Moreover, plasma IL-6 is inversely related to insulin sensitivity in healthy subjects (8). However, in people with type 2 diabetes, the circulating IL-6 concentration is correlated with adipose tissue mass, rather than whole-body insulin sensitivity (9), suggesting that IL-6 may be a marker of obesity without any direct contribution to the development of insulin resistance. IL-6 has also been proposed to enhance glucose uptake and metabolism in skeletal muscle after exercise. During exercise, plasma IL-6 levels increase in an intensity- and duration-dependent manner (10,11). The elevation of plasma IL-6 during exercise is contributed mainly by the contracting skeletal muscle, such that during exercise, skeletal muscle may become the predominant source of IL-6 (10). Thus, IL-6 has been proposed to enhance glucose uptake and metabolism in skeletal muscle after exercise (12). Skeletal muscle plays a paramount role in the regulation of insulin-mediated glucose uptake. Consequently, elevations in plasma IL-6 may directly target skeletal muscle and regulate glucose metabolism.

IL-6 has been reported to have positive and negative actions on metabolic responses in liver, adipose tissue, and skeletal muscle. In cultured hepatocytes IL-6 directly impairs insulin action (13–16). IL-6 impairs differentiation of preadipocytes and reduces insulin action in mature adipocytes (17,18). In contrast, in cultured skeletal muscle cells, insulin action after IL-6 exposure is enhanced (19,20). In vivo infusions of IL-6 in rodent models have also yielded divergent results. Some reports provide evidence that IL-6 infusion in mice reduces insulin action on hepatic glucose output and skeletal muscle glucose uptake (21), whereas others report no effect of IL-6 on whole-body glucose disposal (22). Furthermore, IL-6–deficient ($IL\text{-}6^{-/-}$)

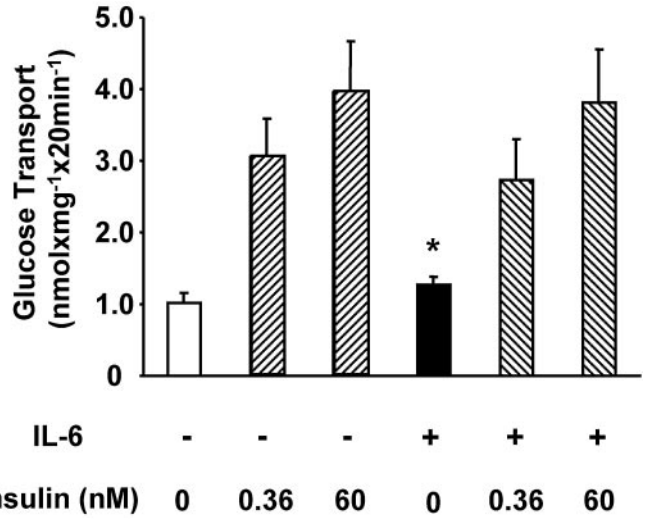

FIG. 1. IL-6 increases glucose transport in human skeletal muscle in vitro. Skeletal muscle strips from eight healthy subjects were incubated with (■) or without (□) 120 ng/ml IL-6. After 30 min, insulin was added to basal (▨) or IL-6–containing media (▧) to a final concentration of either 0.36 or 60 nmol/l as indicated. Glucose transport was determined by accumulation of intracellular 3-*O*-methyl-[^{3}H]glucose. Results are expressed as nanomoles per milligram of protein per 20 min. Results are the means ± SE. *$P < 0.05$ vs. basal.

mice develop obesity at 6–7 months of age, accompanied by disturbances in lipid and carbohydrate metabolism (23).

We recently reported that IL-6 directly enhances glucose uptake and metabolism and enhances insulin sensitivity in cultured myotubes prepared from human skeletal muscle biopsies (20). Signaling pathways via AMP-activated protein kinase (AMPK) (19) and phosphatidylinositol (PI) 3-kinase (20) have been implicated in the effects of IL-6 on glucose metabolism in cultured cells. However, the direct effect of IL-6 on glucose uptake and metabolism in intact human skeletal muscle is unknown. This is a relevant question to resolve, given the clinical and experimental evidence linking IL-6 with the metabolic regulation of glucose homeostasis. Thus, we determined the direct effect of IL-6 on signal transduction and glucose metabolism in human skeletal muscle in vitro. In addition, we assessed whether IL-6 exposure modified insulin action on signal transduction and glucose metabolism.

RESEARCH DESIGN AND METHODS

The study protocol was approved by the ethical committee of Karolinska Institutet. Informed consent was received from all subjects before participation. The clinical characteristics of the healthy male volunteers ($n = 22$) are presented in Table 1. Glucose, insulin, and A1C values were within the normal range, and serum aminotransferases were not elevated. None of the subjects were smokers or reported taking any medication. The subjects were asked to refrain from strenuous exercise for 48 h before the study and to report to the laboratory after an overnight fast.

Muscle biopsy procedure. Skeletal muscle (~1 g) was obtained by means of an open biopsy. Biopsies were taken under local anesthesia (mepivakain chloride 5 mg/ml) from the vastus lateralis portion of the quadriceps femoris (24). Muscle specimens (10 mg) were dissected from the biopsy material, mounted on Plexiglas clips (9 mm in width), and incubated for 30 min in individual flasks containing oxygenated (95% O_2/5% CO_2) Krebs-Henseleit bicarbonate buffer (KHBB) supplemented with 5 mmol/l glucose, 15 mmol/l mannitol, and 0.1% BSA.

Glucose transport. Skeletal muscle was incubated in KHBB supplemented with 5 mmol/l glucose, 15 mmol/l mannitol, and 0.1% BSA. Muscles were preincubated for 30 min in the absence or presence of 120 ng/ml IL-6 (human recombinant; Roche). The IL-6 concentration was maintained throughout all subsequent incubation steps. Thereafter, muscles were incubated in KHBB supplemented with 18 mmol/l mannitol, 2 mmol/l pyruvate, and 0.1% BSA and incubated for 30 min in the absence or presence of 0.36 or 60 nmol/l insulin (Insulin Actrapid, Novo Nordisk). The concentration of insulin was maintained throughout all subsequent incubation steps. Subsequently, skeletal muscle strips were incubated for 20 min in KHBB containing 5 mmol/l 3-*O*-methyl-[^{3}H]glucose (800 μCi/mmol) and 15 mmol/l [^{14}C]mannitol (53 μCi/mmol). At the end of the incubation protocol, skeletal muscle specimens were blotted of excess fluid, snap-frozen in liquid nitrogen, and stored at −80°C until further analysis. Glucose transport was determined by the accumulation of intracellular 3-*O*-methyl-[^{3}H]glucose. Muscle lysate was stored at −80°C for subsequent signal transduction analysis.

TABLE 1
Clinical characteristics of the study participants

Subject characteristics	Means ± SE
Age (years)	48.59 ± 1.79
Weight (kg)	81.67 ± 2.41
Height (m)	1.76 ± 0.01
BMI (kg/m^2)	24.76 ± 0.64
Plasma insulin (pmol/l)	29.88 ± 3.66
Plasma glucose (mmol/l)	5.07 ± 0.10
A1C (%)	4.48 ± 0.05
Total cholesterol (mmol/l)	5.04 ± 0.17
HDL cholesterol (mmol/l)	1.37 ± 0.10
LDL cholesterol (mmol/l)	3.34 ± 0.16
Triglycerides (mmol/l)	0.93 ± 0.13
ASAT (ukat/l)	0.37 ± 0.03
ALAT (ukat/l)	0.46 ± 0.06
GT (ukat/l)	0.46 ± 0.10

ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; GT, γ-glutamyltransferase.

Glucose incorporation into glycogen and oxidation. Muscles were incubated in sealed vials containing 2 ml of oxygenated (95% O_2/5% CO_2) KHBB supplemented with 15 mmol/l mannitol, 0.1% BSA, and 5 mmol/l [U-^{14}C]glucose (0.3 μCi/ml). Muscles were incubated in the absence (basal) or presence of either 120 ng/ml IL-6 or 120 nmol/l insulin. Oxygen supply was stopped after the first 15 min of the incubation period. After 1 h, vials were placed on ice for 3 min. Muscles were quickly removed and frozen between tongs cooled to the temperature of liquid nitrogen. Glucose oxidation measurements were performed as previously described (25). Vials were quickly resealed, and a center well containing 200 μl Protosol (PerkinElmer Life Sciences) was introduced. The media was acidified with 0.5 ml of 15% perchloric acid, and released CO_2 was collected for 1 h. Thereafter, center wells were removed, and [^{14}C] was determined. Glucose incorporation into glycogen was determined as previously described (26). Muscles were weighed and dissolved in 0.5 ml of 1N sodium hydroxide (NaOH) for 30 min. Subsequently, 0.5 ml of 20% trichloric acid was added, and samples were mixed and subjected to centrifugation (3,500*g* at 10°C for 15 min). The supernatant was transferred to a new vial. Thereafter, 200 μl of a glycogen solution (20 mg/ml) and 2 ml of 95% ethanol were sequentially added, and the mixture was incubated at −20°C for 1 h. After centrifugation (15 min at 2,000*g*), the resulting pellet was dissolved in water.

Muscle incubation protocol for signal transduction analysis. To establish a time course for the effects of IL-6 on signal transduction, skeletal muscle was incubated in KHBB supplemented with 5 mmol/l glucose, 15 mmol/l mannitol, and 0.1% BSA. Muscle specimens were incubated for 15 or 30 min in the absence or presence of IL-6. At the end of the incubation period, muscle specimens were blotted of excess fluid, snap-frozen in liquid nitrogen, and stored at −80°C until further analysis. Furthermore, skeletal muscle samples from the glucose transport incubation were also analyzed, and thus a time course of 15-, 30-, and 80-min IL-6 exposure was determined.

Western blot analysis. Total proteins extracted from the muscle strips that were incubated for signal transduction analysis, as well as for the determination of glucose transport, were subjected to Western blot analysis. Muscle specimens were homogenized in 0.3 ml of ice-cold lysis buffer containing 20 mmol/l Tris (pH 8.0), 137 mmol/l NaCl, 2.7 mmol/l KCl, 10 mmol/l NaF, 1 mmol/l MgCl, 1 mmol/l Na_3VO_4, 0.2 mmol/l phenylmethylsulfonyl fluoride, 10% glycerol, 1% Triton X-100, 1 μg/ml aprotinin, 1 μg/ml leupeptin, and 1 nmol/l microcystin using a glass-on-glass system with a motor pestle. Homogenates were solubilized by end-over-end mixing at 4°C for 60 min and subjected to centrifugation (4°C at 12,000*g* for 10 min). The supernatant was stored at −80°C until use. Total protein was determined using a commercially available kit (Pierce, Rockford, IL). Equal amounts of protein (40 μg) were diluted in Laemmli sample buffer. Proteins were separated by SDS-PAGE and trans-

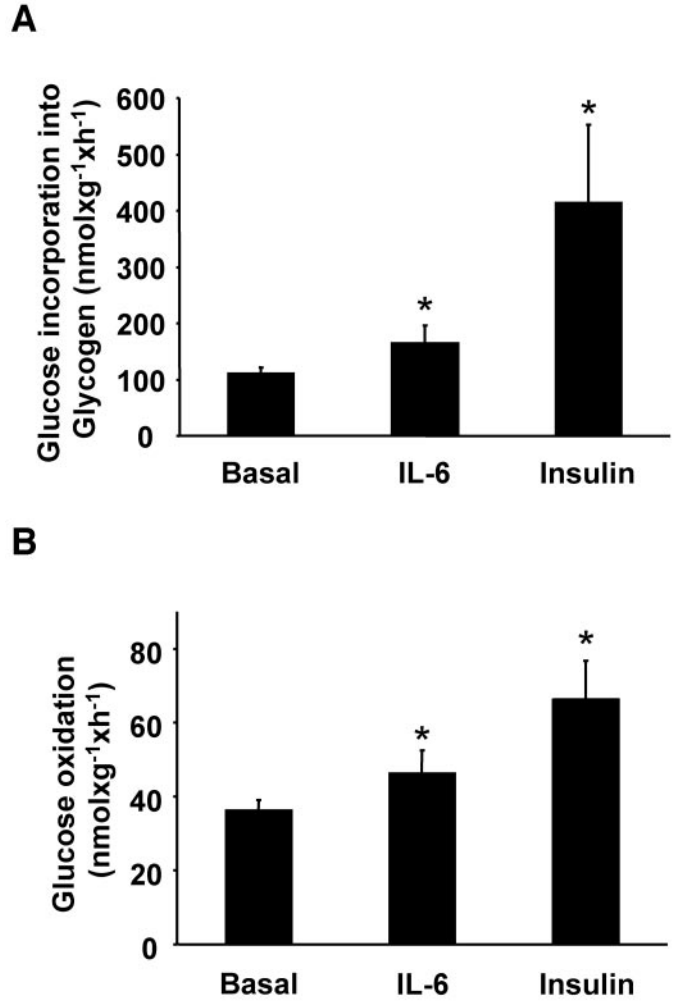

FIG. 2. IL-6 increases glucose metabolism in human skeletal muscle in vitro. Skeletal muscle strips from seven healthy subjects were incubated either without stimulation or with 120 ng/ml IL-6 or 120 nmol/l insulin. *A*: Glucose incorporation into glycogen was measured as the amount of [^{14}C]glucose incorporated into glycogen during 1 h. *B*: Glucose oxidation was measured as the amount of [^{14}C]CO_2 released during 1 hour. Results are the means ± SE. **P* < 0.05 vs. basal in a paired Student's *t* test.

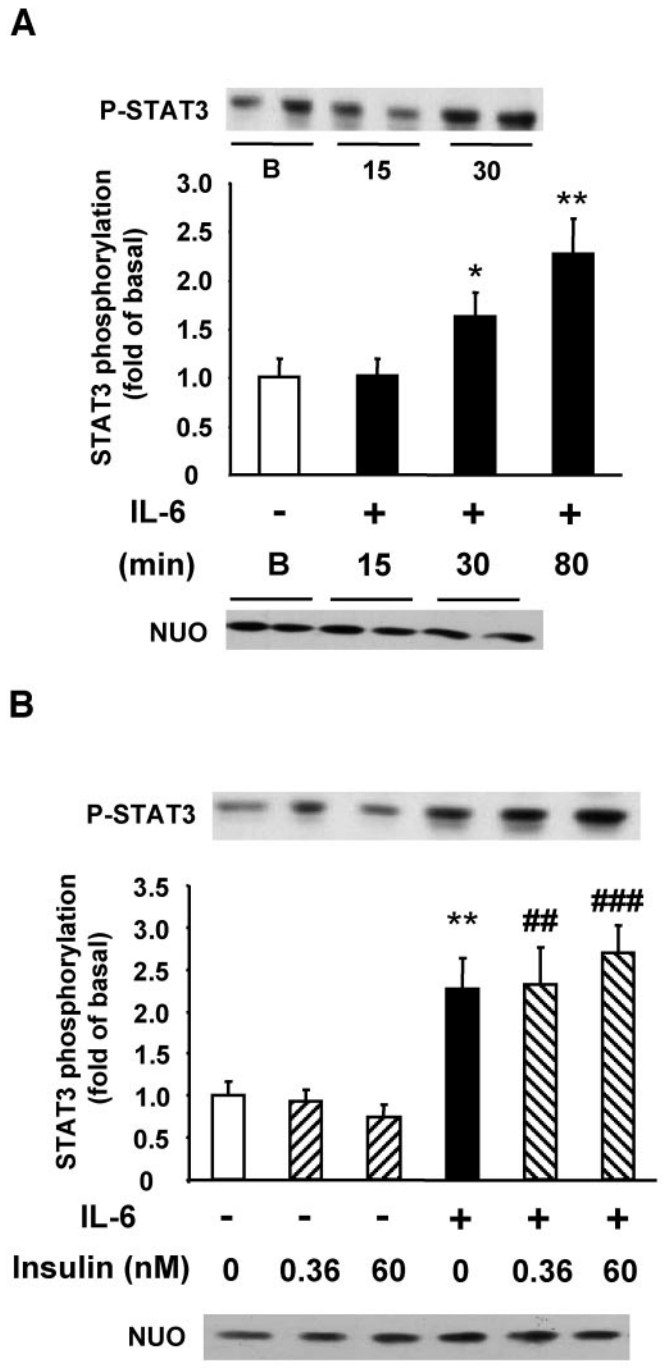

FIG. 3. IL-6 induction of STAT3 signaling in human skeletal muscle in vitro. STAT3 was measured after 15-min (n = 6) and 30-min (n = 14) exposure to IL-6 (*A*), and after 80 min (n = 8) at the end of the glucose transport protocol (*B*). Skeletal muscle strips from healthy subjects were incubated with (■) or without (□) 120 ng/ml IL-6. After 30 min, insulin was added to basal (▨) or IL-6 containing media (▧) to a final concentration of either 0.36 nmol/l or 60 nmol/l as indicated. NADH-ubiquinol oxidoreductase (NUO) protein expression was determined to control for equal loading. Representative blots are shown (*A* and *B*). Results are the means ± SE. **P* < 0.05, ***P* < 0.01 vs. basal; ## *P* = 0.01, ###*P* < 0.001 effect of insulin after prestimulation with IL-6 vs. insulin effect alone.

ferred to Immobilon-P membranes (Millipore, Bedford, MA). Western blot analysis was performed using phosphospecific antibodies against signal transducer and activator of transcription 3 (STAT3; Y705; Upstate), AMPK (Thr^{172}; Cell Signaling), glycogen synthase kinase-α/β (GSK3α/β; Cell Signaling), protein kinase B (PKB)/Akt (Ser^{473}; New England Biolabs), PKB/Akt (Thr^{308}; Cell Signaling), p38 MAPK (Thr^{180}/Tyr^{182}; Cell Signaling), PAS (Cell Signaling), S6 ribosomal protein (Ser$^{235/236}$; Cell Signaling). Equal loading was ensured using a NUO (NADH ubiquinol oxidoreductase) protein–specific antibody (Molecular Probes, Eugene, OR). Proteins were visualized by chemiluminescence and quantified by densitometry.

Phosphatidylinositol 3-kinase activity. An aliquot of the supernatant fraction (800 μg of protein) was immunoprecipitated overnight (4°C) with anti–insulin receptor substrate 1 (IRS-1) antibody coupled to protein A-Sepharose. IRS-1–associated phosphatidylinositol (PI) 3-kinase activity was determined as described previously (27). The reaction products were separated by thin-layer chromatography (Silica Gel 60; Merck, Darmstadt, Germany). The radioactivity incorporated into PI was quantified by a phosphoimager (Image Reader BAS-1800 II; Fujifilm, Düsseldorf, Germany), and the results were normalized to a standard consisting of insulin-stimulated mouse liver.

Restriction fragment–length polymorphism IL-6 gene analysis. Determination of IL-6 polymorphism at −174 was determined as previously described (28). The promoter region surrounding −174 was amplified by PCR using primers as described elsewhere (28). The reaction was carried out in a final volume of 50 μl containing 1.5 mmol/l $MgCl_2$, 0.2 mmol/l of each dNTP, 0.2 mmol/l of sense and anti-sense primer, and 2.5 units *Taq* polymerase. DNA was amplified by an initial denaturation of 10 min at 94°C, 35 cycles consisting of 1 min of denaturation at 94°C, 1 min and 35 s of annealing at 55°C, 1 min of extension at 72°C, and a final extension of 10 min at 72°C. Resultant PCR products were digested with *Sfa*NI restriction enzyme at 37°C overnight and separated on a 2% agarose gel. *Sfa*NI restriction fragment–length polymor-

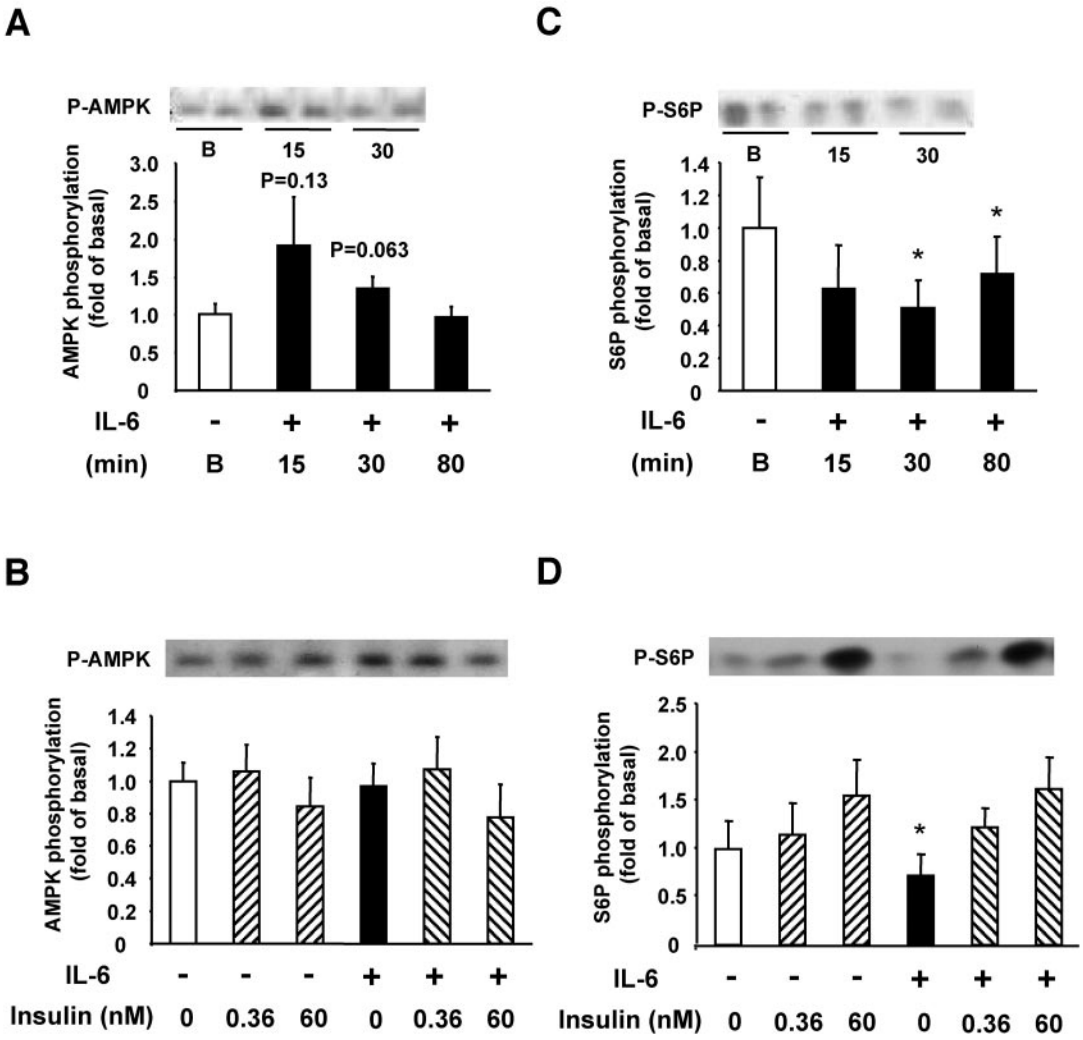

FIG. 4. IL-6 induction of AMPK and S6 ribosomal protein (S6P) signaling in human skeletal muscle in vitro. AMPK and S6 ribosomal protein phosphorylation was measured after 15-min (n = 6) and 30-min (n = 14) exposure to IL-6 (*A* and *C*, respectively), and after 80 min (n = 8) at the end of the glucose transport protocol (*B* and *D*, respectively). Results are the means ± SE. *P < 0.05 vs. basal. Skeletal muscle strips from healthy subjects were incubated with (■) or without (□) 120 ng/ml IL-6. After 30 min, insulin was added to basal (▨) or IL-6 containing media (▧) to a final concentration of either 0.36 nmol/l or 60 nmol/l as indicated.

phism was detected by ethidium bromide staining. Genotypes were classified according to the presence or absence of the enzyme restriction site *Sfa*NI. G/G, G/C, and C/C are homozygous for the presence of the site (140/58 bp), heterozygous for the presence and absence of the site (198/140/58 bp), and homozygous for the absence of the site (198 bp), respectively.

Statistics. Data are the means ± SE. Differences between groups to determine the effect of IL-6 on insulin-stimulated signaling and metabolism were detected by ANOVA followed by Fisher's least significant post hoc analysis. A paired Student's t test was applied when testing the direct effect of IL-6 on signaling and metabolism. Statistical significance was accepted for $P <$ 0.05. As expected, the effect of insulin alone was significant for glucose transport, glucose incorporation into glycogen, glucose oxidation, and PI 3-kinase activity. Insulin alone also increased the phosphorylation of components downstream of the insulin receptor (PKB/Akt, GSK3α/β, AKT substrate of 160 kDa [AS160], and S6 ribosomal protein). Because the effect of insulin was not the scope of this study, only the effects of IL-6 on subsequent signaling are reported.

RESULTS

Subject characteristics. The metabolic characteristics of the subjects are presented in Table 1. All subjects were healthy and had no known first-degree relative with type 2 diabetes. Genotyping was performed on 10 subjects for IL-6 gene promoter polymorphism at position −174. One-third of subjects were heterozygous for GC and the remainder homozygous for GG. None of the subjects were homozygous for the rarer CC allele (the CC allele has been linked to lower IL-6 transcription) (29).

Glucose transport. IL-6 has a direct effect on glucose metabolism in primary cultures established from human skeletal muscle satellite cells (20,30). We determined the effects of IL-6 on glucose uptake and metabolism in intact human skeletal muscle. Muscle strips were incubated in the absence or presence of 120 ng/ml IL-6, and 3-*O*-methyl-glucose transport was assessed. IL-6 increased skeletal muscle glucose transport 1.3-fold ($P < 0.05$) (Fig. 1). As expected, insulin increased glucose transport approximately fourfold ($P < 0.05$). Prestimulation of human muscle strips with IL-6 for 30 min did not alter submaximal (0.36 nmol/l) or maximal (60 nmol/l) insulin-stimulated glucose transport.

Glucose incorporation into glycogen and glucose oxidation. To further assess the fate of glucose after IL-6 stimulation, glucose incorporation into glycogen and glucose oxidation were determined. In isolated human skeletal muscle strips, IL-6 increased glucose incorporation into glycogen 1.5-fold ($P < 0.05$) (Fig. 2*A*). IL-6 increased

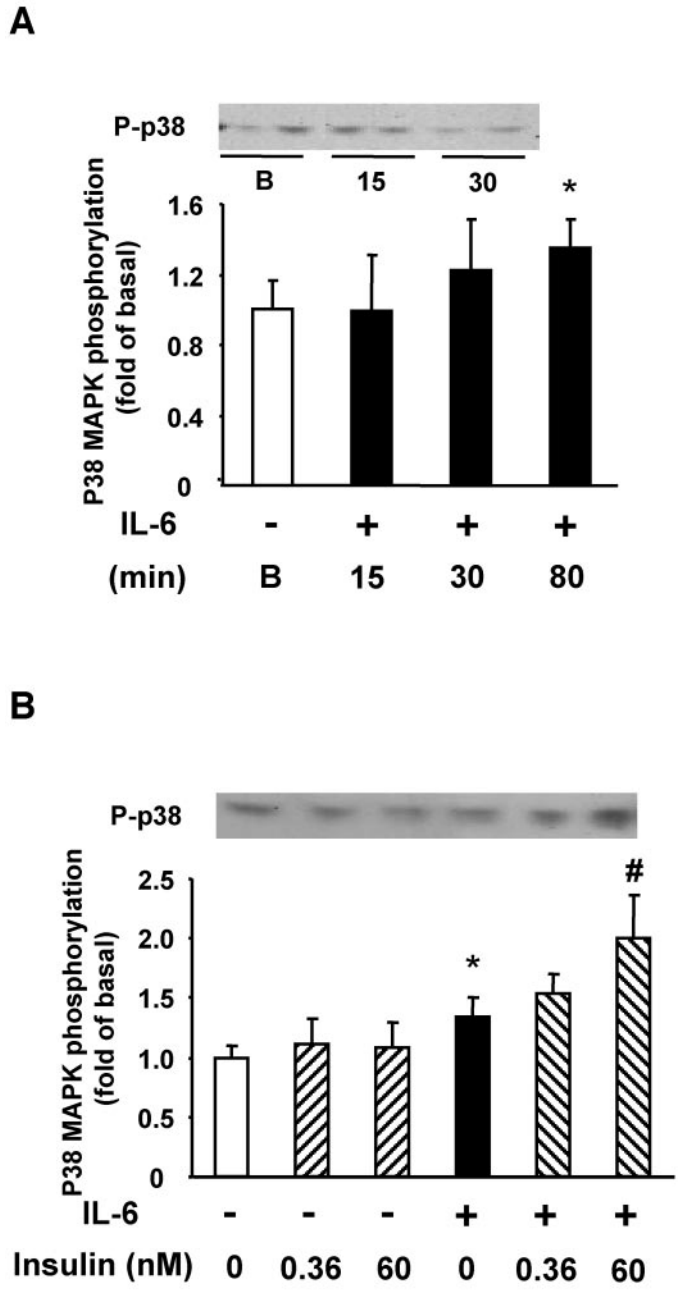

FIG. 5. IL-6 induction of p38 MAPK signaling in human skeletal muscle in vitro. p38 MAPK phosphorylation was measured after 15-min (n = 6) and 30-min (n = 14) exposure to IL-6 (*A*), and after 80 min (n = 8) at the end of the glucose transport protocol (*B*). Skeletal muscle strips from healthy subjects were incubated with (■) or without (□) 120 ng/ml IL-6. After 30 min, insulin was added to basal (▨) or IL-6 containing media (▧) to a final concentration of either 0.36 nmol/l or 60 nmol/l as indicated. Results are the means ± SE. *P < 0.05 vs. basal; #P < 0.05 effect of insulin after prestimulation with IL-6 vs. insulin effect alone.

glucose oxidation 1.3-fold ($P < 0.05$) (Fig. 2*B*). These effects were moderate compared with the effects of insulin (3.7- and 1.8-fold over basal, $P < 0.05$) for glucose incorporation into glycogen and glucose oxidation, respectively (Fig. 2*A* and *B*). In two subjects, the rate of insulin-stimulated glucose incorporation into glycogen in the presence of IL-6 was assessed. Compared with the insulin-stimulated condition, addition of IL-6 resulted in a 1.4- and 1.1-fold increase in glucose incorporation to glycogen for each subject, respectively. Furthermore, in two subjects, coincubation of skeletal muscle with IL-6 and insulin resulted in 0.5- and 0.9-fold increases in glucose oxidation compared with the response to insulin, respectively.

IL-6–mediated intracellular signaling

STAT3. Exposure of human skeletal muscle to IL-6 increased the phosphorylation of STAT3 (Fig. 3*A* and *B*), and maximum effect was observed at 80 min. Under insulin-stimulated conditions, the effect of IL-6 on STAT3 phosphorylation was unaltered.

AMPK. Although there was a strong trend for increased AMPK phosphorylation after 15 and 30 min of IL-6 exposure, this effect did not reach statistical significance (P = 0.13 and P = 0.063, respectively) (Fig. 4*A*). However, when interindividual variation was corrected by calculating IL-6–mediated AMPK phosphorylation as a fold over each individual subject's basal level, IL-6 increased AMPK phosphorylation 2 ± 0.4–fold at 15 min (P = 0.066) and 1.6 ± 0.3–fold at 30 min (P = 0.029). AMPK phosphorylation peaked at 15 min, and levels were restored to basal levels after 80 min of IL-6 stimulation. Insulin had no effect on AMPK phosphorylation, either with or without pre-exposure to IL-6 (Fig. 4*B*).

S6 ribosomal protein. In line with the effect of IL-6 on AMPK phosphorylation, the phosphorylation of S6 ribosomal protein was decreased in response to IL-6 (Fig. 4*C*). However, IL-6 did not affect insulin-induced phosphorylation of S6 ribosomal protein (Fig. 4*D*).

p38 MAPK. Phosphorylation of p38 MAPK was increased after 80 min in muscle strips exposed to IL-6 either alone or in combination with 60 nmol/l insulin ($P < 0.05$) (Fig. 5*A* and *B*).

PI 3-kinase activity and PKB/Akt signaling. In primary human skeletal muscle cells, IL-6–mediated glucose metabolism is dependent on signal transduction via PI 3-kinase (20). Here, we show that in intact human muscle, IRS-1–associated PI 3-kinase activity is unaltered in response to IL-6 stimulation (Fig. 6*A*). Furthermore, IL-6 was without effect on phosphorylation of Akt, AS160, and GSK3α/β (data not shown), and it did not modify the effect of insulin signaling to these proteins (Fig. 6*B–D*; GSK3α/β data not shown).

DISCUSSION

The role of IL-6 in whole-body insulin sensitivity is unresolved (4). Divergent results may be partly caused by distinct effects of IL-6 in different tissues, as well as species differences between humans and rodents. For example, mouse and human IL-6 share only 42% amino acid sequence identity (31). In humans, the plasma IL-6 concentration is inversely related to insulin sensitivity (8). This correlation highlights IL-6 as a biomarker of insulin resistance. In rodents, IL-6 depletion selectively improves hepatic insulin action in obesity (32), providing a direct link between IL-6 and insulin action. Collectively, these studies raise the possibility that IL-6 may contribute to the manifestation of insulin resistance. However, in primary human skeletal muscle cultures, as well as in rodent skeletal muscle cell lines, IL-6 directly enhances glucose metabolism (19,20,30,33). These effects appear to be mediated via PI 3-kinase and PKB/Akt signaling pathways. To delineate the acute effect of IL-6 on glucose metabolism in humans, we examined the direct effects of recombinant human IL-6 on basal and insulin-mediated signal transduction and glucose metabolism in intact skeletal muscle.

Exposure of isolated human skeletal muscle to IL-6

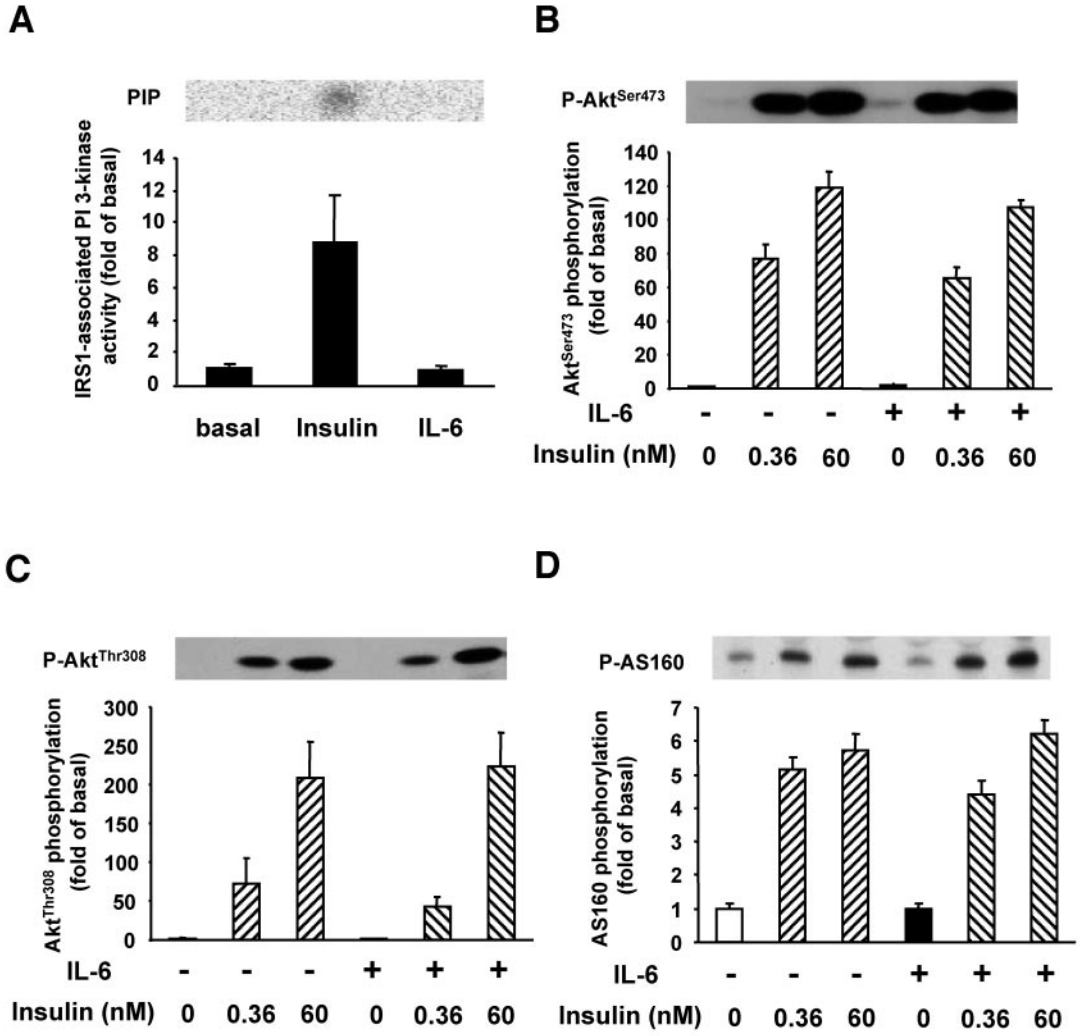

FIG. 6. IL-6 induction of PI 3-kinase signaling in human skeletal muscle in vitro. Skeletal muscle strips from healthy subjects were incubated with (■) or without (□) 120 ng/ml IL-6. After 30 min, insulin was added to basal (▨) or IL-6 containing media (▧) to a final concentration of either 0.36 nmol/l or 60 nmol/l as indicated. IRS-1–associated PI 3-kinase activity was determined as the amount of [γ-^{32}P] incorporated into phosphatidylinositol (*A*). The effect of IL-6 on basal and insulin-stimulated PKB/Akt Thr^{308} (*B*), PKB/Akt Ser^{473} (*C*), and AS160 (*D*) phosphorylation was measured at the end of the glucose transport protocol (n = 8).

modestly enhanced glucose metabolism, as evidenced by increased glucose transport and oxidation, as well as glycogenesis. Recent evidence has highlighted a role for AMPK in mediating metabolic effects of IL-6 (19,20,34). AMPK phosphorylation is important for IL-6–mediated glucose uptake (19) and lipid oxidation (20). The IL-6 effect on AMPK phosphorylation in human skeletal muscle reported here is similar to effects noted in rat muscle (34). Thus, the increased glucose uptake and metabolism noted in the current study are likely to be mediated via AMPK. The increase in AMPK phosphorylation is concomitant with a decreased phosphorylation of S6 ribosomal protein, a downstream target of p70 S6 kinase. This is consistent with the notion that activation of AMPK suppresses protein synthesis through concurrent repression of mTOR (mammalian target of rapamycin) signaling and activation of MAPK signaling (35–37). Interestingly, in intact human muscle, IL-6 effects on glucose metabolism and AMPK phosphorylation are modest and about equal in magnitude.

In primary cultures of human skeletal muscle, as well as rat L6 muscle cells, IL-6 exposure increases PI 3-kinase activity (20,33) and phosphorylation of PKB/Akt (20,30). In contrast, in intact human skeletal muscle, IL-6 was without effect on either PI 3-kinase or PKB/Akt. IL-6–mediated effects on glucose metabolism observed in cultured muscle are of greater magnitude than the effects reported here in intact human skeletal muscle. Furthermore, in intact human muscle, IL-6 effects on glucose metabolism are not additive with those of insulin, again in contrast to results in cultured muscle (20,30). This may in fact reflect a fundamental difference between the response of cultured muscle derived from skeletal muscle satellite cells and intact adult human muscle to IL-6. We have previously shown that IL-6 exposure enhanced growth and differentiation of human satellite cells (20). Thus, the release of IL-6 from contracting skeletal muscle during exercise may elicit signaling events leading to enhanced recruitment of satellite cells and stimulate growth. Mice with a targeted deletion of IL-6 do not have an overt reduction in overall skeletal muscle mass, but they have reduced endurance capacity (38). Furthermore, IL-6 null mice also have impaired tendon healing (39). Targeted overexpression of IL-6 increases the extent of myogenic differentiation and myotube developments in C2C12 cells, which is partly

mediated via activation of p38 MAPK (40). Thus, IL-6 may be an exercise-mediated signal to facilitate muscle growth and repair. The exercise-induced release of IL-6 from skeletal muscle decreases with physical training (41), indicating that IL-6 may facilitate adaptive responses to training. One part of this adaptation may be an increase in IL-6 receptor expression and enhanced skeletal muscle IL-6 sensitivity with training (42).

Despite differences in magnitude of IL-6 effects on glucose metabolism noted between cultured and intact human skeletal muscle, in both systems IL-6 enhances glucose uptake and utilization. In healthy humans, an in vivo infusion of IL-6 results in an ~15% increased glucose infusion rate and increased glucose oxidation, without affecting the suppression of endogenous glucose production during a hyperinsulinemic-euglycemic clamp (19). This is in line with our finding of an ~30% increase in glucose transport into skeletal muscle, the major target tissue for whole-body glucose disposal. An IL-6–mediated increase in glucose uptake and utilization may be of importance during exercise, when circulating insulin levels are low. This could be of particular importance for satellite cells located between the skeletal muscle fibers, which do not contract during exercise and therefore may not activate AMPK. The rapid increase in AMPK phosphorylation by IL-6 may facilitate the effects on glucose metabolism, whereas IL-6 effects on STAT3 and p38 MAPK may represent more sustained effects on gene expression and mediate exercise-induced muscle remodeling.

There is a substantial body of evidence linking IL-6 to different states of insulin resistance. Increased plasma levels of IL-6, together with other cytokines, have been linked to the development of type 2 diabetes (43). IL-6 is also elevated in several clinical situations of inflammation, such as after surgery, during a viral infection, and with autoimmune conditions (44–46). Although these observations are correlative, IL-6 directly induces insulin resistance in vitro in adipocytes (17). In contrast, in skeletal muscle cells, IL-6 appears to have an insulin-sensitizing effect (20,30). This is in line with recent findings showing that exercise-induced IL-6 release from human muscle is positively correlated to exercise intensity and muscle glucose uptake (47). Although exercise increases skeletal muscle IL-6 release, the average circulating serum content of IL-6 from various sources is unrelated to insulin sensitivity, either before or after 1 month of physical training (48). Thus, IL-6 appears to have tissue-specific effects on glucose metabolism.

IL-6 stimulates its own production in skeletal muscle (49), and exercise also leads to increased IL-6 production from the contracting muscle (11,50). The presence of a G allele at position −174 in the promoter is linked to enhanced IL-6 gene expression (29,51), possibly because of a change in a transcription factor binding (51). In the current study, the degree of IL-6 response noted and the genotype at position −174 were not correlated. Furthermore, there were no homozygous carriers of the rarer C allele. The impact of functional polymorphisms in IL-6 action has yet to be established.

In summary, we show that IL-6 directly increases glucose metabolism in intact human skeletal muscle. The enhanced glucose uptake and metabolism is coincident with an increase in AMPK phosphorylation. In contrast to results from isolated satellite cells, IL-6 exposure was without effect on PI 3-kinase and PKB/Akt. Acute IL-6 exposure did not alter the magnitude of insulin-induced phosphorylation of PKB/Akt or ribosomal S6 protein. Collectively, our data provide evidence that in intact human skeletal muscle, acute IL-6 exposure enhances basal glucose metabolism and does not alter insulin sensitivity.

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