Activation of AMP-Activated Protein Kinase by Interleukin-6 in Rat Skeletal Muscle

Association With Changes in cAMP, Energy State, and Endogenous Fuel Mobilization

Meghan Kelly, Marie-Soleil Gauthier, Asish K. Saha, and Neil B. Ruderman

OBJECTIVE—Interleukin-6 (IL-6) directly activates AMP-activated protein kinase (AMPK) in vivo and in vitro; however, the mechanism by which it does so is unknown.

RESEARCH DESIGN AND METHODS—We examined this question in skeletal muscle using an incubated rat extensor digitorum longus (EDL) muscle preparation as a tool.

RESULTS—AMPK activation by IL-6 coincided temporally with a nearly threefold increase in the AMP:ATP ratio in the EDL. The effects of IL-6 on both AMPK activity and energy state were inhibited by coincubation with propranolol, suggesting involvement of β-adrenergic signaling. In keeping with this notion, IL-6 concurrently induced a transient increase in cAMP, and its ability to activate AMPK was blocked by the adenyl cyclase inhibitor 2′5′-dideoxyadenosine. In addition, like other β-adrenergic stimuli, IL-6 increased glycogen breakdown and lipolysis in the EDL. Similar effects of IL-6 on AMPK, energy state, and cAMP content were observed in C2C12 myotubes and gastrocnemius muscle in vivo, indicating that they were not unique to the incubated EDL.

CONCLUSIONS—These studies demonstrate that IL-6 activates AMPK in skeletal muscle by increasing the concentration of cAMP and, secondarily, the AMP:ATP ratio. They also suggest that substantial increases in IL-6 concentrations, such as those that can result from its synthesis by muscles during exercise, may play a role in the mobilization of fuel stores within skeletal muscle as an added means of restoring energy balance. ***Diabetes*** **58:1953–1960, 2009**

Proinflammatory effects of interleukin-6 (IL-6) have been well documented (rev. in 1). In light of this and the presence of elevated plasma levels (two- to threefold) of IL-6 in patients with obesity, diabetes, and atherosclerotic cardiovascular disease (2,3), IL-6 has previously been viewed as a contributor to the development of these and other disorders associated with insulin resistance and the metabolic syndrome. However, studies in humans have demonstrated that IL-6 may have other functions. IL-6 is synthesized and released in large quantities from contracting skeletal muscle, resulting in plasma concentrations 50- to 100-fold higher than those seen at rest (rev. in 4). Furthermore, when IL-6 was infused into humans to achieve plasma levels similar to those seen with intense sustained exercise, it stimulated both lipolysis and fat oxidation (5), and no evidence of insulin resistance was observed. Cumulatively, these findings have led to the suggestion that muscle-derived IL-6 plays a role in regulating mammalian fuel homeostasis during exercise.

AMP-activated protein kinase (AMPK) is a fuel sensing enzyme that responds to cellular energy deficits by increasing catabolic processes that generate ATP (e.g., fatty acid oxidation) and downregulating anabolic processes that consume ATP but are not acutely required for cell survival (e.g., protein, glycerolipid, and cholesterol synthesis) (rev. in 6). The current view is that the primary mechanism by which AMPK is activated is through alterations in cellular energy state, as manifested by increases in the AMP:ATP ratio (rev. in 7). Hormones such as adrenaline (8), leptin (9), and ciliary neurotrophic factor (CNTF) (10) and pharmacological agents such as isoproterenol (11), thiazolidenediones (12), and metformin (13) have been reported to alter cellular energy state and activate AMPK in various tissues.

In addition to hormones and pharmacological agents, a number of studies have demonstrated that exercise also induces AMPK activity in multiple tissues (14). Previously, we have demonstrated that exercise increases AMPK activity in liver and adipose tissue, as well as muscle, in the rat leading to the notion that a systemic factor may be involved. In turn, the finding that increases in AMPK during exercise are markedly attenuated in these tissues in IL-6$^{-/-}$ mice suggested that one such factor could be IL-6 (15). The concurrent demonstrations that IL-6 can directly activate AMPK in skeletal muscle and adipose tissue, both in vivo and in vitro, also supported this notion (15,16). Despite these observations, the mechanism by which IL-6 activates AMPK in various tissues is not known. In the present study, we examined this question in skeletal muscle. The results indicate that IL-6 activates AMPK in an incubated rat extensor digitorum longus (EDL) preparation by increasing the concentrations of cAMP and, secondarily, the AMP:ATP ratio. They also suggest that IL-6 concurrently stimulates lipolysis, glycogenolysis, and fatty acid oxidation in this tissue.

RESEARCH DESIGN AND METHODS

Antibodies for P-AMPK (Thr^{172}), P-STAT3 (Tyr^{705}) cAMP-responsive element binding protein (P-CREB; Ser^{133}), and phosphorylated glycogen synthase

From the Department of Medicine, Section of Endocrinology, Diabetes Research Unit, Boston University School of Medicine, Boston, Massachusetts.
Corresponding author: Neil B. Ruderman, nrude@bu.edu.
Received 18 September 2008 and accepted 20 May 2009.
Published ahead of print at http://diabetes.diabetesjournals.org on 5 June 2009. DOI: 10.2337/db08-1293.

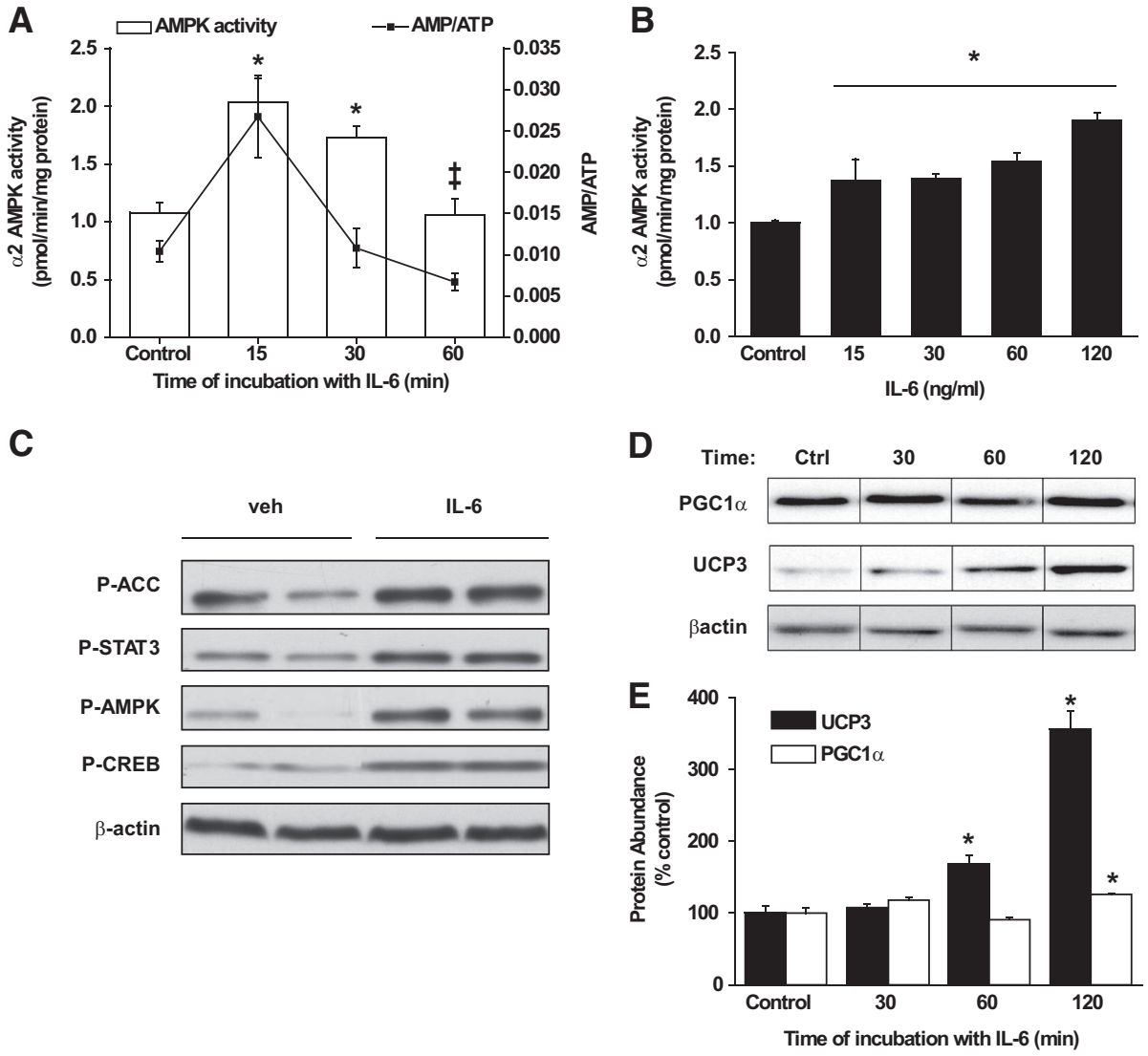


FIG. 1. Incubation with IL-6 increases α2 AMPK activity, protein phosphorylation, and UCP3 and PGC1α protein abundance in incubated EDL muscle. *A*: α2 AMPK activity (bar graph) and AMP:ATP ratio (line graph) were assayed in rat EDL muscles incubated with IL-6 (120 ng/ml) for 15–60 min. *B*: Dose-curve of α2 AMPK activity in response to IL-6 (15–120 ng/ml) for 30 min. *C*: Immunoblots of P-ACC (Ser^{79}), P-STAT3 (Tyr^{705}), P-AMPK (Thr^{172}), P-CREB (Ser^{133}), and β-actin in EDL muscles incubated with IL-6 (120 ng/ml) for 15 min. *D*: Immunoblots of PGC1α, UCP3, and β-actin in EDL incubated with IL-6 (120 ng/ml) for 30–120 min. *E*: Densitometric analysis of immunoblots of UCP3 and PGC1α protein shown in *D*. Results are means ± SE, $n = 4$–10. EDL muscles. *$P < 0.05$ versus control. ‡$P < 0.05$ versus 15 min IL-6. Immunoblots shown are representative of four samples.

kinase 3β (P-GSK3β; Ser^{9}) were obtained from Cell Signaling (Danvers, MA); phosphorylated acetyl CoA carboxylase (P-ACC; Ser^{79}) from Upstate Biotechnologies (Charlottesville, VA); total GSK3β from BD Transduction Laboratories (San Jose, CA); and uncoupling protein 3 (UCP3) from Chemicon (Billerica, MA). Peroxisome proliferator–activated receptor-γ coactivator 1α (PGC1α) antibody and 2′5′ dideoxyadenosine were purchased from Calbiochem (San Diego, CA), rat recombinant IL-6 from Pierce Biotechnologies (Rockford, IL), and Protein A/G PLUS agarose beads from Santa Cruz Biotechnology (Santa Cruz, CA). "SAMS" peptide and polyclonal antibodies that immunoprecipitate the α1 or the α2 catalytic subunit of AMPK and were used for activity assays were obtained from QCB Biotechnology (Hopkinton, MA). Dulbecco's modified Eagle's medium (DMEM), GlutMAX, penicillin/streptomycin, and horse serum were purchased from Invitrogen (Carlsbad, CA) and FBS from Hyclone (Logan, UT). All other materials were purchased from Sigma Aldrich (St. Louis, MO).

Sprague-Dawley rats (50–100 g), obtained from Charles River Laboratories (Framingham, MA), were housed in the Boston University School of Medicine Animal Facility. Animals were on a 12-h light/dark cycle and were allowed standard rodent diet and water ad libitum. Food was withdrawn 16–20 h before experiments. All studies were conducted according to the Institutional Animal Care and Use Committee (IACUC) at Boston University.

In vitro experiments. On the experimental day, rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and EDL muscles were removed and tied to stainless steel clips as described previously (17). Muscles were preincubated for 20 min at 37°C in oxygenated (95% O_2/5% CO_2) Krebs-Henseleit solution containing 6 mmol/l glucose and then incubated in the absence or presence of rat recombinant IL-6 (15–120 ng/ml) for the times indicated (10–120 min). For palmitate oxidation measurements, muscles were incubated with or without IL-6 (120 ng/ml) in the presense of 0.2 mmol/l palmitate complexed to 2% BSA in media containing 0.2 μCi/ml [U-^{14}C] palmitate. After 30 min, media was acidified with acetic acid (0.5 N) and the $^{14}CO_2$ released was trapped on hyamine hydroxide–soaked filter paper. At the end of the incubation protocols, muscles were blotted, quick-frozen in liquid nitrogen, and stored at −80°C until additional analyses were performed.

In vivo experiments. Male Sprague-Dawley rats weighing 100 g (±5 g) were used for experiments in which they were injected with IL-6 (25 ng/g animal weight i.p.). Sixty minutes after the injection, animals were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and whole gastrocnemius muscle was excised, frozen in liquid nitrogen, and stored at −80°C until further analysis.

Cell culture experiments. C2C12 myoblasts were purchased from American *Type Culture* Collection (Rockville, MD). The cells were placed in six-well plates and cultured in DMEM containing 1% GlutMAX, 1% penicillin/streptomycin, and 10% FBS. When myoblasts reached 80% confluence, their differentiation to myotubes was induced by switching the media to DMEM containing 2% horse serum, 1% GlutMAX, and 1% penicillin/streptomycin. Experiments were performed when over 80% of cells had formed myotubes.

TABLE 1
IL-6 alters intracellular concentrations of adenine nucleotides and creatine phosphate

	Time of incubation (min)				
	Control	10	15	30	60
ATP (μmol/g tissue)	4.4 ± 0.2	3.8 ± 0.3	3.2 ± 0.3*	4.1 ± 0.4	4.1 ± 0.2†
ADP (μmol/g tissue)	0.67 ± 0.02	0.84 ± 0.04	0.73 ± 0.12	0.67 ± 0.04	0.83 ± 0.11
AMP (μmol/g tissue)	0.04 ± 0.004	0.045 ± 0.003	0.07 ± 0.006*	0.05 ± 0.007	0.03 ± 0.007†
Creatine phosphate (μmol/g tissue)	13.9 ± 0.5	10.9 ± 0.5*	10.7 ± 0.8*	12.5 ± 1.0	12.9 ± 0.4†

Data are means ± SE (n = 5-8). Rat EDL muscles were incubated with IL-6 (120 ng/ml) for 10–60 min. Because no significant differences in the nucleotide content were observed in the control muscles at the different time points, values for control muscles at all time points were pooled for statistical purposes. *$P < 0.05$ vs. control, †$P < 0.05$ vs. 15 min IL-6.

AMPK activity. AMPK activity was assayed in frozen skeletal muscle homogenized in lysis buffer as described previously (18). Briefly, muscle lysate containing 200 μg protein was immunoprecipitated with antibody specific to the α2 or α1 catalytic subunit of AMPK and protein A/G agarose beads. Beads were washed five times, and the activity of the immobilized enzyme was assayed based on the phosphorylation of "SAMS" peptide (0.2 mmol/l) by 0.2 mmol/l ATP (containing 2 μCi [γ-^{32}P] ATP) in the presence and absence of 0.2 mmol/l AMP. Label incorporation into the SAMS peptide was measured on a Racbeta 1214 scintillation counter.

Western blotting. Cultured cells were scraped on ice in cell lysis buffer (Cell Signaling Technology, Beverly, MA) (plus 1 mmol/l phenylmethylsulfonyl fluoride) and centrifuged (14,000*g* for 15 min at 4°C). Protein concentrations of cell supernatants were determined with the bicinchoninic acid reagents (Pierce, Rockford, IL) using BSA as the standard. Fifty micrograms of protein lysate from skeletal muscle or C2C12 cells were run on a 4–15% gradient polyacrylamide gel and transferred onto a polyvinylidene fluoride membrane. Membranes were then stained with Ponceau S (1% in 5% acetic acid) to ensure even transfer and blocked in Tris-buffered saline (pH 7.5) containing 0.05% Tween 20 (TBST) and 5% milk for 1 h at room temperature. Blots were first incubated overnight in primary antibody diluted in TBST containing 5% BSA (1:1,000 for P-AMPK, P-ACC, P-GSK3β, T-GSK3β; 1:2,000 for P-STAT3 and PGC1α; and 1:500 for P-CREB and UCP3) and then in TBST containing 5% nonfat dry milk and the appropriate secondary antibody conjugated to horseradish peroxidase at a 1:5,000 dilution. After this, the membranes were subjected to an enhanced chemiluminescence solution, and multiple autoradiogaphs (to ensure linearity) were generated. Densitometry was performed using Scion Image software.

Adenine nucleotides and cAMP measurements. Concentrations of ATP, ADP, AMP, and phosphocreatine were initially determined spectrophotometrically in neutralized perchloric acid filtrates as described previously (19). cAMP content was determined with a commercially available enzyme-linked immunosorbent assay kit according to the instructions provided by the manufacturer (Alpco Diagnostics). Samples assayed for cAMP were digested in 5% trichloroacetic acid (TCA), and the supernatant was extracted five times in an equal volume of diethyl ether. The aqueous layer was dried using the SpeedVac sc110, and pellets were stored at −80°C until analysis. On the day of analysis, pellets were reconstituted in 250 μl of deionized water. In studies in which both cAMP and adenine nucleotides were assayed, tissues were extracted with TCA.

Other measurements. Lactate release into the incubation medium was determined spectrophotometrically as described previously (20), and glycerol release was determined with a commercially available kit after concentration of the medium by lyophilization (Sigma). Tissue glycogen content was determined using the phenol-sulfuric acid reaction (21).

Statistical analyses. Results are expressed as means ± SE. Statistical significance was determined by a one-way or two-way ANOVA for nonrepeated measures, as appropriate. The Bonferroni post hoc test was used in the event of a significant ($P < 0.05$) ratio.

RESULTS

IL-6 activates α2 AMPK in intact rat skeletal muscle. The time course and dose response of AMPK activation by IL-6 are presented in Fig. 1*A* and *B*. Incubation of EDL muscle with IL-6 (120 ng/ml) has been shown previously to induce a twofold increase in the phosphorylation of the α subunit of AMPK at Thr^{172} (an index of its activity) after 15 min, with values returning to baseline by 60 min (15). As shown in Fig. 1*A*, an almost identical pattern was observed when the activity of the immunoprecipitated α2 isoform of AMPK was measured. In contrast, no change in the activity of the α1 isoform of AMPK was observed (data not shown). Activation of the α2 AMPK occurred at IL-6

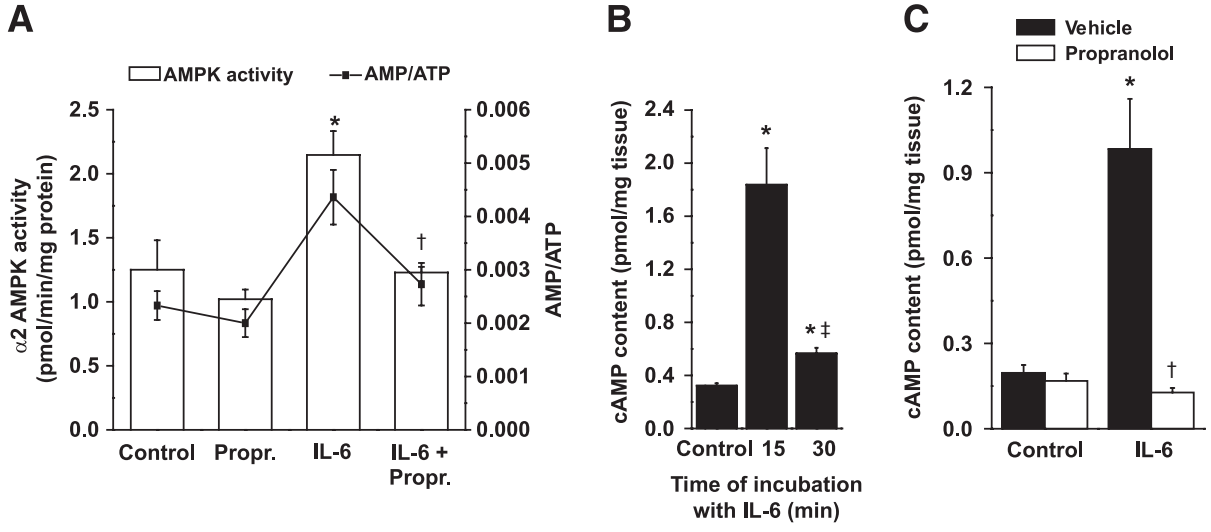


FIG. 2. IL-6–induced increases in α2 AMPK activity, cAMP levels, and AMP:ATP ratio are abrogated by propanolol (Propr). *A*: Activity of α2 AMPK (bar graph) and AMP:ATP ratio (line graph) in EDL pretreated or not pretreated for 30 min with the β-adrenergic antagonist propranolol (10 μmol/l) and then with or without IL-6 (120 ng/ml) for 15 min. *B*: cAMP levels in EDL muscles incubated with IL-6 (120 ng/ml) for 15 or 30 min. *C*: cAMP levels in EDL preincubated with or without propranolol (10 μmol/l) for 30 min and then with or without IL-6 (120 ng/ml) for 15 min. Results are means ± SE (n = 4–7), *$P < 0.05$ versus control, no propanalol, †$P < 0.05$ versus IL-6 no propanalol, ‡$P < 0.05$ versus 15-min time point.

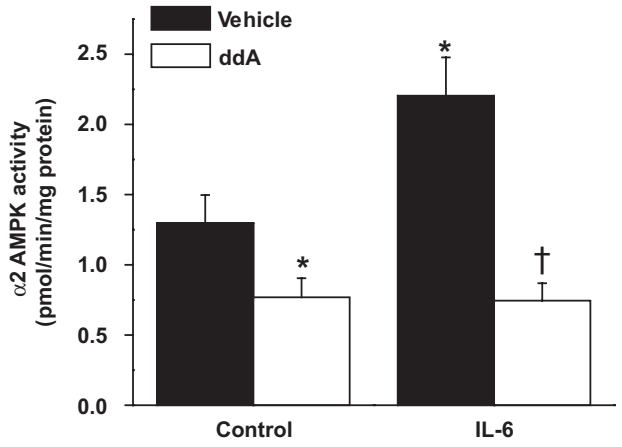
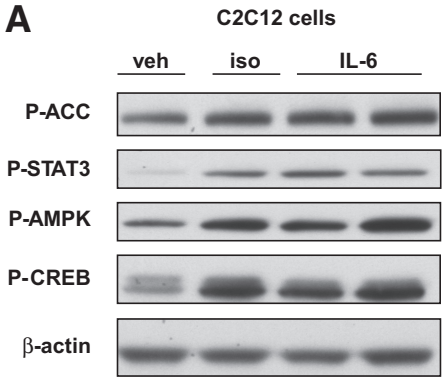


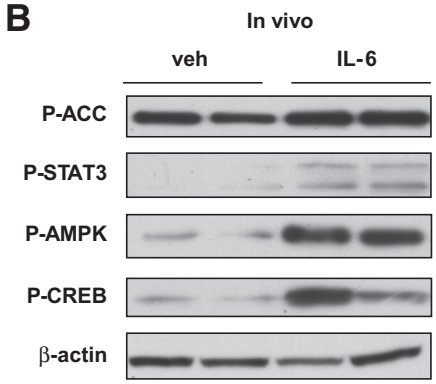
FIG. 3. The adenylyl cyclase inhibitor 2′-5′ dideoxyadenosine (ddA) blocks IL-6-induced α2 AMPK activity. Activity of α2 AMPK in EDL pretreated (■) or not pretreated (□) with dideoxyadenosine (100 μmol/l) for 30 min and then with or without IL-6 (120 ng/ml) for 15 min. Results are means ± SE (n = 4–6), *P < 0.05 versus control, no inhibitor, †P < 0.05 versus IL-6 no inhibitor.

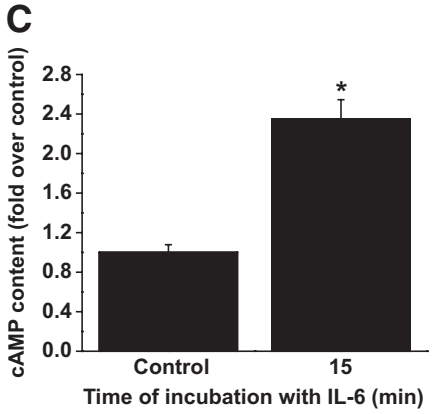
concentrations as low as 15 ng/ml; however, it was somewhat less than that observed at 120 ng/ml (Fig. 1*B*). Incubation with IL-6 (120 ng/ml) for 15 min increased the phosphorylation of AMPK (Thr 172) and of its downstream target ACC (Ser[79]) (Fig. 1*C*) in keeping with the changes in AMPK α2 activity. Thereafter, P-AMPK and P-ACC returned to baseline values after 60 min and remained so for up to 4 h, the maximum length of incubation tested (data not shown). IL-6 also increased the phosphorylation of STAT3 (Tyr 705), a known event in the activation of the JAK/STAT signaling cascade (22) (Fig. 1*C*). Finally, although the increase in AMPK activity was transient, it was followed by two- and fourfold increases in UCP3 protein abundance after 1 and 2 h, respectively (Fig. 1*D* and *E*). A smaller increase in PGC1α protein expression (25%) was observed; however, it only achieved statistical significance after 2 h (Fig. 1*D* and *E*).

IL-6 alters the levels of intracellular adenine nucleotides. As a first step in studying the mechanism by which IL-6 activates AMPK, we examined its effect on cellular energy state. Control muscles gave similar values to those reported previously (23); however, incubation with IL-6 (120 ng/ml) led to decreases in the concentrations of creatine phosphate and ATP, no significant changes in ADP, and an increase in AMP levels (Table 1). This resulted in increases in the AMP:ATP ratio that paralleled those of AMPK activity, with both of them being maximal at 15 min and returning to baseline by 60 min (Fig. 1*A*).

The effects of IL-6 on AMPK activity are dependent on cAMP production and energy state alterations. Koh et al. (8) have reported that the injection of adrenaline activates AMPK and increases the AMP:ATP ratio in rat adipose tissue and that these changes are inhibited by the β-adrenergic antagonist propranolol. Here we found that propranolol, at a concentration of 10 μmol/l, also inhibited IL-6–induced AMPK activation, raising the possibility that







D

Adenine Nucleotide and cAMP Content (μmol/g tissue)		
	control	IL-6
ATP	5.16 ± 0.1	4.46 ± 0.06*
ADP	0.26 ± 0.02	0.24 ± 0.01
AMP	0.025 ± 0.001	0.46 ± 0.009*
AMP:ATP	0.005 ± 0.0003	0.010 ± 0.002*
cAMP	0.0013 ± 0.00012	0.0066 ± 0.0024

FIG. 4. IL-6 exerts similar effects in C2C12 myotubes and gastrocnemius muscle in vivo. Immunoblots of P-ACC (Ser[79]), P-STAT3 (Tyr[705]), P-AMPK (Thr[172]), P-CREB (Ser[133]), and β-actin were performed on (*A*) lysates of C2C12 myotubes treated with IL-6 (50 ng/ml) or isoproterenol (iso) (10 μmol/l) for 30 min and (*B*) homogenates of gastrocnemius muscle of rats injected intraperitoneally with saline (veh) or 2.5 μg of IL-6 1 h before being killed. Immunoblots shown are representative of n = 3. *C*: cAMP levels assayed in extracts of C2C12 myotubes treated for 15 min with IL-6 (50 ng/ml). *D*: Adenine nucleotide and cAMP levels in muscles studied in *B*. Results are means ± SE, n = 3–4. *P < 0.05 versus control.

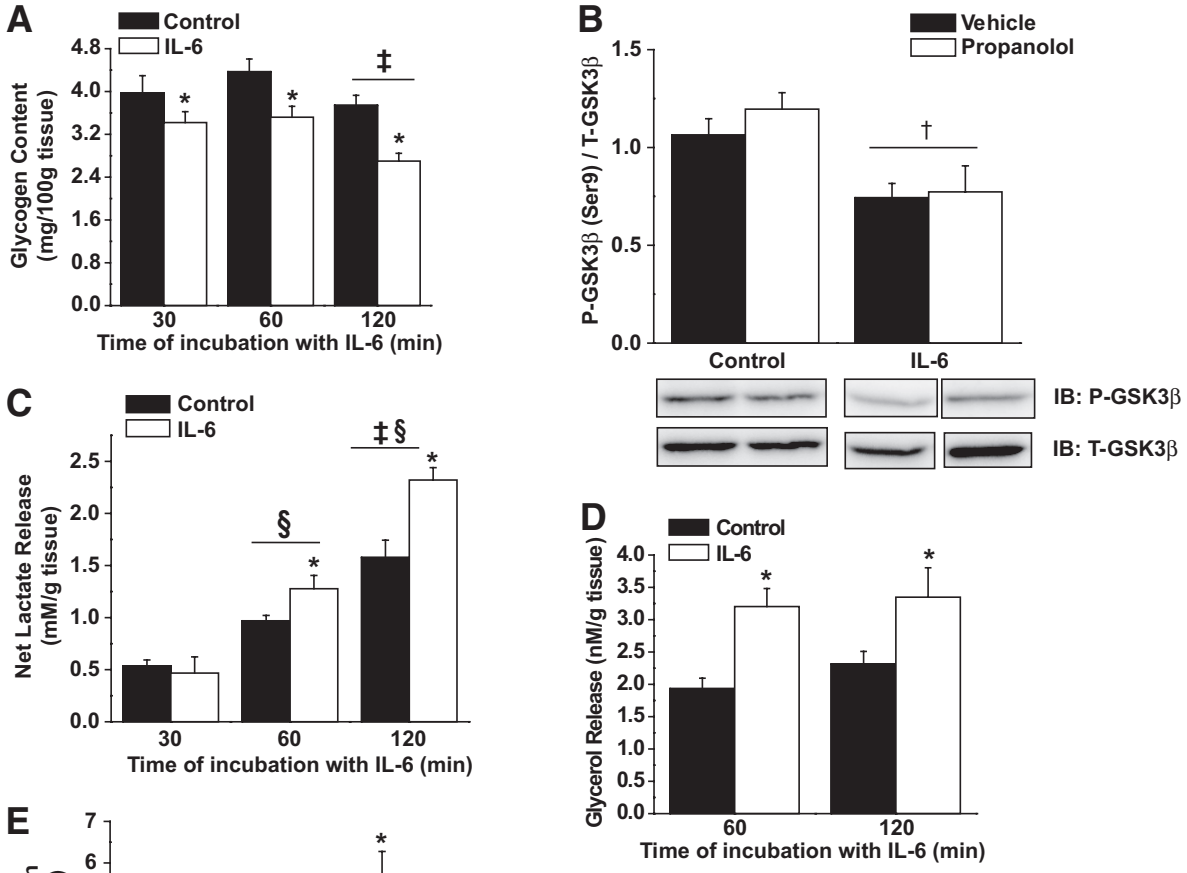


FIG. 5. Effects of IL-6 on substrate mobilization and metabolism in incubated EDL muscle. Incubated EDL muscles were treated with (□) or without (■) IL-6 (120 ng/ml) for times indicated. *A*: Glycogen content. *B*: Representative immunoblot blots and densitometric analysis of P-GSK3β and T-GSK3β. *C* and *D*: Release into the medium of lactate and glycerol. *E*: Fatty acid oxidation in EDL incubated with IL-6 (120 ng/ml) in the presence of 0.2 μCi/ml ^{14}C-palmitate and 0.2 mmol/l palmitate bound to fatty acid–free albumin for 30 min. Palmitate oxidation was quantified by measuring the concentration of $^{14}CO_2$ released into the medium over the next 30 min (see RESEARCH DESIGN AND METHODS). Results are means ± SE, n = 4–9. *P < 0.05 and †P < 0.01 versus control, ‡P < 0.05 versus 60 min IL-6, §P < 0.05 versus 30 min IL-6.

IL-6 caused β-adrenergic stimulation (Fig. 2*A*). In keeping with this notion, 15-min incubation with IL-6 (120 ng/ml) induced an increase in both cAMP levels and the phosphorylation of its downstream target P-CREB at Ser^{133} (Figs. 2*B* and 1*C*). As expected, the IL-6–induced increase in cAMP was prevented by propranolol (Fig. 2*C*). In addition, propranolol prevented the increases in the AMP:ATP ratio caused by IL-6 (Fig. 2*A*). As shown in Fig. 1*A*, the alterations in the AMP:ATP ratio (Fig. 2*A*, line-graph) coincided with changes in α2 AMPK activity (Fig. 2*A*, bar graph). Finally, consistent with these results, activation of AMPK by IL-6 was also prevented by the adenyl cyclase inhibitor 2′5′ dideoxyadenosine (Fig. 3).

The effects of IL-6 on energy state and cAMP are also observed in cultured C2C12 myotubes and gastrocnemius muscle in vivo. Treatment of C2C12 cells with IL-6 increased cAMP, P-ACC, P-AMPK, P-STAT3, and P-CREB abundance, as did isoproterenol (Fig. 4*A* and *C*), suggesting both that the effects of IL-6 on the EDL are not unique to the incubated muscle and that they can be mimicked by isoproterenol, a known β-adrenergic agonist. In keeping with the former conclusion, IL-6 injected intraperitoneally for 60 min also led to increases in the abundance of P-ACC, P-AMPK, P-STAT3, and P-CREB and in the AMP:ATP ratio in the gastrocnemius muscle, just as it did in the incubated muscle (Fig. 4*B* and *D*).

IL-6 increases lipolysis and glycogenolysis. IL-6 has been reported to increase whole-body lipolysis (5) and decrease glycogen content in primary hepatocytes (24), as do catecholamines, glucagon, and other agents that increase cAMP. For this reason, we assessed whether IL-6 had similar effects on muscle. As shown in Fig. 5*A*, incubation of the EDL with IL-6 (120 ng/ml) for 120 min diminished muscle glycogen by 30%. In keeping with this, IL-6 treatment decreased the phosphorylation of GSK3β suggesting that it inhibited glycogen synthase activity (Fig. 5*B*). (Phosphorylase A and glycogen synthase activity per se were not assayed.) The decrease in GSK3β phosphorylation was not affected by propranolol, indicating that it is independent of AMPK activation. IL-6 also caused a 1.5-fold increase in lactate release into the media and increased the release of glycerol, suggesting that glycolysis and lipolysis were also stimulated by this treatment (Fig. 5*C* and *D*). Finally, in agreement with previous studies in cultured myotubes and soleus muscle (25), incubation of the EDL for 30 min with IL-6 caused a fivefold increase in

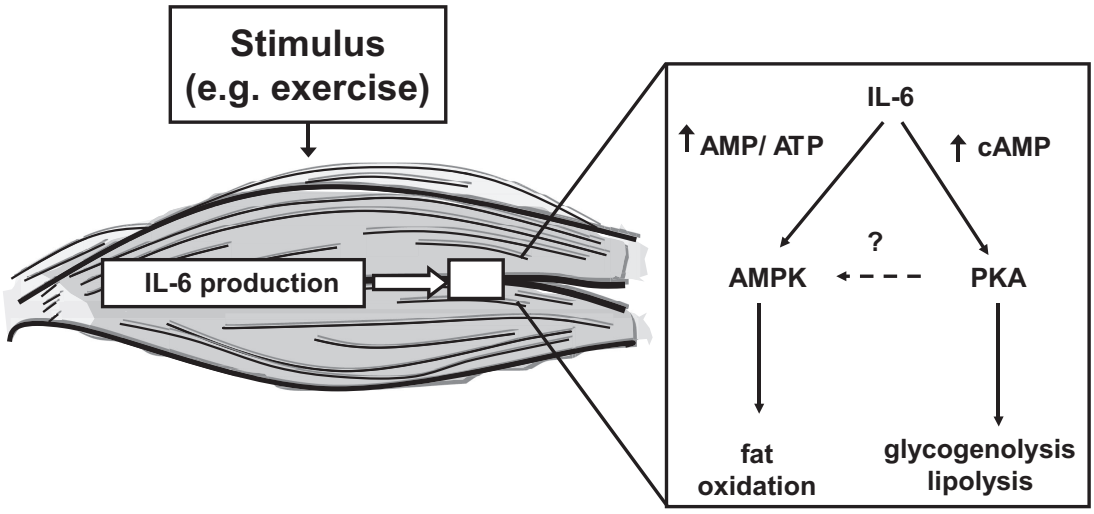


FIG. 6. Paracrine functions of IL-6 synthesized and released by muscle during exercise. During sustained intense exercise, IL-6 is synthesized and released from skeletal muscle cells and increases muscle cAMP, AMP:ATP ratio, and AMPK activity. This results in enhanced muscle fatty acid oxidaton, lipolysis, and glycogenolysis.

palmitate oxidation (Fig. 5*E*), an effect attributed to AMPK activation.

DISCUSSION

IL-6 acutely activates AMPK in skeletal muscle both in vivo and in vitro (15,16,25,26). The results of the present study indicate that it does so by altering events that increase intracellular cAMP and, secondarily, the AMP:ATP ratio. In support of this notion, IL-6–induced AMPK activation in the incubated EDL was accompanied by increases in cAMP, and this, as well as the increase in the AMP:ATP ratio, was completely inhibited by the β-adrenergic antagonist propranolol. Additionally, the adenyl cyclase inhibitor 2′5′ dideoxyadenosine blocked the activation of AMPK by IL-6. Finally, IL-6 caused similar changes in C2C12 myotubes and gastrocnemius muscle in vivo, indicating its effects were not unique to the incubated EDL.

How the IL-6–induced elevation of cAMP in turn increased the AMP:ATP ratio remains to be determined. Another agent that increases cAMP in skeletal muscle, isoproterenol, also has been reported to increase AMPK activity (27), lipolysis, and glycogenolysis (28); however, its effect on energy state was not examined. On the other hand, such measurements have been carried out in adipose tissue. Investigations in 3T3-L1 adipocytes have demonstrated that increases in the AMP:ATP ratio and AMPK activation occur when cAMP is increased by either forskolin or isoproterenol (11) and that they are dependent on the stimulation of lipolysis. Studies by Koh et al. (8) have suggested that a similar mechanism occurs in rat adipose tissue in vivo, in that exercise and adrenaline (epinephrine)-induced increases in the AMP:ATP ratio and AMPK activity were both inhibited by propranolol. Recently, it has been reported that IL-6 induces lipolysis in porcine adipocytes and that this effect appears to be dependent on the actions of IL-6 on ERK1/2 and its ability to directly phosphorylate hormone-sensitive lipase (HSL) (29); however, the role of AMPK in this setting was not examined. Whether the stimulation of lipolysis accounts for the IL-6–induced changes in energy state and AMPK activity in the EDL muscle as it does in adipose tissue remains to be determined.

It has been clearly demonstrated that cAMP and protein kinase A (PKA) activation increase the biosynthesis of IL-6 in various cells (30); however, IL-6–induced increases in cAMP have not been previously reported. Thus, one major question is, how does IL-6 induce cAMP production? We speculate that an intermediary molecule could be protein kinase C-δ (PKCδ), given that this enzyme mediates the activation of the cAMP producing adenyl cyclase isoform VII (31) and that IL-6 induces its membrane translocation in hepatocytes (32). Also, PKCδ mediates the binding of the IL-6 transmembrane receptor gp130 to STAT3 (33,34), and it has been implicated in IL-6–induced insulin signaling (34).

The effects of IL-6 on the phosphorylation of CREB (Ser^{133}) have not been reported previously; however, it has been noted that both AMPK and PKA phosphorylate CREB at the same residue (Ser^{133}) (35). In addition, both AMPK (36,37) and CREB (38) appear to regulate the abundance of UCP3 and PGC1α. Whether AMPK or PKA is responsible for the IL-6–induced increases in UCP3 and PGC1α observed in the present study remains to be determined experimentally. It is noteworthy that these effects of IL-6 are qualitatively similar to those produced by leptin and CNTF (10,39), both of which also induce a rapid and transient activation of AMPK, increase the AMP:ATP ratio (9,10), and activate the JAK/STAT signaling cascade (22). Recently, it has been reported that STAT3 can localize in mitochondria and enhance oxidative phosphorylation (40), suggesting that it could directly influence cellular ATP levels.

In previous reports, a concentration of IL-6 (120 ng/ml) well above that in plasma was used to induce AMPK activation in incubated rat skeletal muscle (15,26,41). In the present study, AMPK activation was observed at IL-6 concentrations as low as 15 ng/ml; however, this is still significantly higher than the reported resting concentration of IL-6 in the plasma of a rat (10–300 pg/ml) (42,43). During sustained exercise, the muscle cell is most likely exposed to much higher concentrations of IL-6 given that it is synthesizing IL-6 and releasing it into the circulation at amounts sufficient to increase its plasma concentration dramatically. Thus, in humans, IL-6 release from muscle during exercise can result in plasma concentrations 50- to 100-fold higher than those at rest (44) and concentrations

in the interstitial fluid of muscle 10- to 100-fold higher than those in plasma (45,46). In one study, an interstitial fluid IL-6 concentration of 1.2 ng/ml was observed in humans after low-grade repetitive exercise without a detectible change in plasma IL-6 (46). Therefore, in the rat, if one assumes a low (~10 pg/ml) resting plasma IL-6 level and an effect of exercise similar to that in humans, an interstitial fluid IL-6 concentration between 10–100 ng/ml would be achieved during intense exercise; this, however, remains to be determined.

It has been proposed that IL-6 synthesized and released into the circulation by skeletal muscle during exercise is an endocrine signal from the muscle cell that is becoming fuel deficient (4). In keeping with this notion, IL-6 increases adipose tissue lipolysis and hepatic glycogenolysis (during exercise) in humans (5,47) and its release from muscle is enhanced when muscle glycogen is depleted (48). As already noted, large increases in muscle interstitial fluid IL-6 have been observed in humans after low-intensity exercise in the absence of a change in plasma IL-6. This, together with the results of the present study, suggest that IL-6 could also act as an autocrine or paracrine factor that enhances lipolysis and glycogenolysis and activates AMPK in the muscle cell during exercise (Fig. 6). Finally, the physiological relevance of IL-6 in this setting is strongly suggested by the observation that both the ability to sustain exercise and activate AMPK are impaired in IL-6$^{-/-}$ mice (15,49,50).

In summary, the results indicate that IL-6, at concentrations hypothetically representative of those released during exercise, activates AMPK in skeletal muscle by increasing cAMP production and, secondarily, the AMP:ATP ratio. They also indicate that this transient activation of AMPK results in a more sustained increase in the protein expression of two AMPK-mediated genes that act on mitochondria, PGC1α, and UCP3. Finally, they reveal that IL-6 increases substrate availability within the muscle cell by increasing glycogenolysis and lipolysis. Studies in IL-6$^{-/-}$ mice have established that a lack of IL-6 markedly inhibits AMPK activation during exercise (15), whether it also diminishes the increases in cAMP levels, and PGC1α and UCP3 expression caused by exercise, remains to be determined.

ACKNOWLEDGMENTS

This study was supported by USPHS Grants RO1DK19514 and RO1DK067509 (N.R.). M.K. was supported by Ruth L. Kirschstein NRSA Postdoctoral Training Grant HL-07224 and M.S.G. by a postdoctoral research fellowship from Fonds de la recherche en santé du Québec (FRSQ).

No potential conflicts of interest relevant to this article were reported.

The authors thank Kathleen Tumelty, Vaishali Patel, and Drs. José Cacicedo, Eva Tomas, and Keith Tornheim for valuable advice and technical assistance.

Portions of this study were presented in poster form at the American Physiological Society conference "The Integrated Biology of Exercise V," Hilton Head, South Carolina, 24–27 September 2008.

REFERENCES

1. Naka T, Nishimoto N, Kishimoto T. The paradigm of IL-6: from basic science to medicine. Arthritis Res 2002;4(Suppl.):S233–S242
2. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3–L1 adipocytes and is, like IL-8 and tumor necrosis factor-alpha, overexpressed in human fat cells from insulin-resistant subjects. J Biol Chem 2003;278:45777–45784
3. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab 2001;280:E745–E751
4. Febbraio MA, Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB J 2002;16:1335–1347
5. van Hall G, Steensberg A, Sacchetti M, Fischer C, Keller C, Schjerling P, Hiscock N, Moller K, Saltin B, Febbraio MA, Pedersen BK. Interleukin-6 stimulates lipolysis and fat oxidation in humans. J Clin Endocrinol Metab 2003;88:3005–3010
6. Kahn BB, Alquier T, Carling D, Hardie DG. AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab 2005;1:15–25
7. Hardie DG, Scott JW, Pan DA, Hudson ER. Management of cellular energy by the AMP-activated protein kinase system. FEBS Lett 2003;546:113–120
8. Koh HJ, Hirshman MF, He H, Li Y, Manabe Y, Balschi JA, Goodyear LJ. Adrenaline is a critical mediator of acute exercise-induced AMP-activated protein kinase activation in adipocytes. Biochem J 2007;403:473–481
9. Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 2002;415:339–343
10. Watt MJ, Dzamko N, Thomas WG, Rose-John S, Ernst M, Carling D, Kemp BE, Febbraio MA, Steinberg GR. CNTF reverses obesity-induced insulin resistance by activating skeletal muscle AMPK. Nat Med 2006;12:541–548
11. Gauthier MS, Miyoshi H, Souza SC, Cacicedo JM, Saha AK, Greenberg AS, Ruderman NB. AMP-activated protein kinase (AMPK) is activated as a consequence of lipolysis in the adipocyte: potential mechanism and physiological relevance. J Biol Chem 2008;283:16514–16524
12. LeBrasseur NK, Kelly M, Tsao TS, Farmer SR, Saha AK, Ruderman NB, Tomas E. Thiazolidinediones can rapidly activate AMP-activated protein kinase in mammalian tissues. Am J Physiol Endocrinol Metab 2006;291:E175–E181
13. Zhang L, He H, Balschi JA. Metformin and phenformin activate AMP-activated protein kinase in the heart by increasing cytosolic AMP concentration. Am J Physiol Heart Circ Physiol 2007;293:H457–H466
14. Park H, Kaushik VK, Constant S, Prentki M, Przybytkowski E, Ruderman NB, Saha AK. Coordinate regulation of malonyl-CoA decarboxylase, sn-glycerol-3-phosphate acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. J Biol Chem 2002;277:32571–32577
15. Kelly M, Keller C, Avilucea PR, Keller P, Luo Z, Xiang X, Giralt M, Hidalgo J, Saha AK, Pedersen BK, Ruderman NB. AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. Biochem Biophys Res Commun 2004;320:449–454
16. Ruderman NB, Keller C, Richard AM, Saha AK, Luo Z, Xiang X, Giralt M, Ritov VB, Menshikova EV, Kelley DE, Hidalgo J, Pedersen BK, Kelly M. Interleukin-6 regulation of AMP-activated protein kinase. Potential role in the systemic response to exercise and prevention of the metabolic syndrome. Diabetes 2006;55(Suppl. 1):S48–S54
17. Saha AK, Kurowski TG, Ruderman NB. A malonyl-CoA fuel-sensing mechanism in muscle: effects of insulin, glucose, and denervation. Am J Physiol 1995;269:E283–E289
18. Vavvas D, Apazidis A, Saha AK, Gamble J, Patel A, Kemp BE, Witters LA, Ruderman NB. Contraction-induced changes in acetyl-CoA carboxylase and 5′-AMP-activated kinase in skeletal muscle. J Biol Chem 1997;272:13255–13261
19. Lowry O, Passoneau J. *A Flexible System for Enzymatic Analysis*. New York, Academic Press, 1972, p. 147–156
20. Gutmann I, Wahlefeld A. L(+)-Lactate. Determination with lactate dehydrogenase and NAD. Bergmeyer H, Ed. *Methods of Enzymatic Analysis*. New York, Academic, 1974, p. 1464–1468
21. Lo S, Russell JC, Taylor AW. Determination of glycogen in small tissue samples. J Appl Physiol 1970;28:234–236
22. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 2003;374:1–20
23. Itani SI, Saha AK, Kurowski TG, Coffin HR, Tornheim K, Ruderman NB. Glucose autoregulates its uptake in skeletal muscle: involvement of AMP-activated protein kinase. Diabetes 2003;52:1635–1640
24. Kanemaki T, Kitade H, Kaibori M, Sakitani K, Hiramatsu Y, Kamiyama Y, Ito S, Okumura T. Interleukin 1β and interleukin 6, but not tumor necrosis factor alpha, inhibit insulin-stimulated glycogen synthesis in rat hepatocytes. Hepatology 1998;27:1296–1303
25. Carey AL, Steinberg GR, Macaulay SL, Thomas WG, Holmes AG, Ramm G, Prelovsek O, Hohnen-Behrens C, Watt MJ, James DE, Kemp BE, Pedersen BK, Febbraio MA. Interleukin-6 increases insulin-stimulated glucose dis-

posal in humans and glucose uptake and fatty acid oxidation in vitro via AMP-activated protein kinase. Diabetes 2006;55:2688–2697
26. Glund S, Deshmukh A, Long YC, Moller T, Koistinen HA, Caidahl K, Zierath JR, Krook A. Interleukin-6 directly increases glucose metabolism in resting human skeletal muscle. Diabetes 2007;56:1630–1637
27. Ruderman NB, Park H, Kaushik VK, Dean D, Constant S, Prentki M, Saha AK. AMPK as a metabolic switch in rat muscle, liver and adipose tissue after exercise. Acta Physiol Scand 2003;178:435–442
28. Alam N, Saggerson ED. Malonyl-CoA and the regulation of fatty acid oxidation in soleus muscle. Biochem J 1998;334(Pt 1):233–241
29. Yang Y, Ju D, Zhang M, Yang G. Interleukin-6 stimulates lipolysis in porcine adipocytes. Endocrine 2008;33:261–269
30. Rohrbach S, Engelhardt S, Lohse MJ, Werdan K, Holtz J, Muller-Werdan U. Activation of AP-1 contributes to the beta-adrenoceptor-mediated myocardial induction of interleukin-6. Mol Med 2007;13:605–614
31. Nelson EJ, Hellevuo K, Yoshimura M, Tabakoff B. Ethanol-induced phosphorylation and potentiation of the activity of type 7 adenylyl cyclase: involvement of protein kinase C Δ. J Biol Chem 2003;278:4552–4560
32. Suh HN, Lee SH, Lee MY, Lee YJ, Lee JH, Han HJ. Role of interleukin-6 in the control of DNA synthesis of hepatocytes: involvement of PKC, p44/42 MAPKs, and PPARΔ. Cell Physiol Biochem 2008;22:673–684
33. Novotny-Diermayr V, Zhang T, Gu L, Cao X. Protein kinase C delta associates with the interleukin-6 receptor subunit glycoprotein (gp) 130 via Stat3 and enhances Stat3-gp130 interaction. J Biol Chem 2002;277:49134–49142
34. Weigert C, Hennige AM, Lehmann R, Brodbeck K, Baumgartner F, Schauble M, Haring HU, Schleicher ED. Direct cross-talk of interleukin-6 and insulin signal transduction via insulin receptor substrate-1 in skeletal muscle cells. J Biol Chem 2006;281:7060–7067
35. Thomson DM, Herway ST, Fillmore N, Kim H, Brown JD, Barrow JR, Winder WW. AMP-activated protein kinase phosphorylates transcription factors of the CREB family. J Appl Physiol 2008;104:429–438
36. Zhou M, Lin BZ, Coughlin S, Vallega G, Pilch PF. UCP-3 expression in skeletal muscle: effects of exercise, hypoxia, and AMP-activated protein kinase. Am J Physiol Endocrinol Metab 2000;279:E622–E629
37. Jager S, Handschin C, St-Pierre J, Spiegelman B. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1α. Proc Natl Acad Sci U S A 2007;104:12017–12022
38. Branvold DJ, Allred DR, Beckstead DJ, Kim HJ, Fillmore N, Condon BM, Brown JD, Sudweeks SN, Thomson DM, Winder WW. Thyroid hormone effects on LKB1, MO25, phospho-AMPK, phospho-CREB, and PGC-1α in rat muscle. J Appl Physiol 2008;105:1218–1227
39. Tajima D, Masaki T, Hidaka S, Kakuma T, Sakata T, Yoshimatsu H. Acute central infusion of leptin modulates fatty acid mobilization by affecting lipolysis and mRNA expression for uncoupling proteins. Exp Biol Med (Maywood) 2005;230:200–206
40. Wegrzyn J, Potla R, Chwae YJ, Sepuri NB, Zhang Q, Koeck T, Derecka M, Szczepanek K, Szelag M, Gornicka A, Moh A, Moghaddas S, Chen Q, Bobbili S, Cichy J, Dulak J, Baker DP, Wolfman A, Stuehr D, Hassan MO, Fu XY, Avadhani N, Drake JI, Fawcett P, Lesnefsky EJ, Larner AC. Function of mitochondrial Stat3 in cellular respiration. Science 2009;323:793–797
41. Geiger PC, Hancock C, Wright DC, Han DH, Holloszy JO. IL-6 increases muscle insulin sensitivity only at superphysiological levels. Am J Physiol Endocrinol Metab 2007;292:E1842–E1846
42. Prestes J, de Ferreira CK, Dias R, Frollini AB, Donatto FF, Cury-Boaventura MF, Guereschi MG, Pithon-Curi TC, Verlengia R, Palanch AC, Curi R, Cavaglieri CR. Lymphocyte and cytokines after short periods of exercise. Int J Sports Med 2008;29:1010–1014
43. Gadonski G, LaMarca BB, Sullivan E, Bennett W, Chandler D, Granger JP. Hypertension produced by reductions in uterine perfusion in the pregnant rat: role of interleukin 6. Hypertension 2006;48:711–716
44. Steensberg A, Febbraio MA, Osada T, Schjerling P, van Hall G, Saltin B, Pedersen BK. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. J Physiol 2001;537:633–639
45. Langberg H, Olesen JL, Gemmer C, Kjaer M. Substantial elevation of interleukin-6 concentration in peritendinous tissue, in contrast to muscle, following prolonged exercise in humans. J Physiol 2002;542:985–990
46. Rosendal L, Sogaard K, Kjaer M, Sjogaard G, Langberg H, Kristiansen J. Increase in interstitial interleukin-6 of human skeletal muscle with repetitive low-force exercise. J Appl Physiol 2005;98:477–481
47. Febbraio MA, Hiscock N, Sacchetti M, Fischer CP, Pedersen BK. Interleukin-6 is a novel factor mediating glucose homeostasis during skeletal muscle contraction. Diabetes 2004;53:1643–1648
48. Keller C, Steensberg A, Hansen AK, Fischer CP, Plomgaard P, Pedersen BK. Effect of exercise, training, and glycogen availability on IL-6 receptor expression in human skeletal muscle. J Appl Physiol 2005;99:2075–2079
49. Wallenius V, Wallenius K, Ahren B, Rudling M, Carlsten H, Dickson SL, Ohlsson C, Jansson JO. Interleukin-6-deficient mice develop mature-onset obesity. Nat Med 2002;8:75–79
50. Faldt J, Wernstedt I, Fitzgerald SM, Wallenius K, Bergstrom G, Jansson JO. Reduced exercise endurance in interleukin-6-deficient mice. Endocrinology 2004;145:2680–2686