ARTICLE

Contraction-Induced Myokine Production and Release: Is Skeletal Muscle an Endocrine Organ?

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FEBBRAIO, M.A., and B.K. PEDERSEN. Contraction-induced myokine production and release: Is skeletal muscle an endocrine organ? *Exerc. Sport Sci. Rev.*, Vol. 33, No. 3, pp. 114–119, 2005. *The concentration of plasma interleukin-6 (IL-6) increases during physical exercise, but until recently the cellular origin of this increase has been unknown. Recent work has identified that skeletal muscle is a major source of this increase and the release of IL-6 from muscle can mediate metabolic processes. IL-6 is, therefore, the first identified "myokine" released from muscle that can now be termed an endocrine organ.* **Key Words:** interleukin-6, metabolic processes, cytokines, hepatic glucose production, lipolysis

INTRODUCTION

The role of cytokines in health and disease has received growing interest with the recent discovery that the adipose tissue is an endocrine organ capable of secreting substances to modulate metabolic processes. One "adipokine" (a cytokine secreted from adipose tissue) that is known to modulate metabolic processes is the pleiotropic multifunctional cytokine interleukin-6 (IL-6). It has been known for some years that plasma concentrations of IL-6 rise markedly during physical activity, but the origin of this increase has only become apparent recently. Over the past 5 yrs, work from our laboratories has been able to demonstrate that IL-6 is produced within contracting skeletal muscle cells and then released into the circulation, and in this respect we can now refer to IL-6 as a myokine (a cytokine secreted from active skeletal muscle). Moreover, we have been able to show that contraction-induced increases in IL-6 can result in activation of antiinflammatory pathways and augment endogenous glucose production and glucose clearance. These latter data provide the first evidence that the skeletal muscle can release substances capable of modulating metabolic processes. This evidence that the skeletal muscle is an endocrine organ opens the door for future studies that examine the biological importance of "myokinemia," skeletal muscle–derived increases in plasma cytokines.

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SOURCES OF CONTRACTION-INDUCED INCREASES IN CIRCULATING IL-6

It has been well documented that the plasma concentration of IL-6 increases up to more than 100-fold during muscular exercise. This increase is followed by the appearance of cytokine inhibitors such as IL-1 receptor antagonist (IL-1ra) and the antiinflammatory cytokine IL-10, but it is clear that with regard to contraction-induced alterations, circulating IL-6 are comparatively most remarkable (5). Only until recently has the source of this IL-6 been identified. Because it was commonly thought that the exercise-induced increase in IL-6 was a consequence of an immune response caused by local damage in the working muscles, it was assumed that either circulating immune cells and/or the liver may be potential cells/organs that contribute to the exercise-induced increase in IL-6. Work from our group demonstrated that monocytes are not the source of the exercise-induced increase in plasma IL-6. Using flow cytometric techniques, we showed that the number, percentage, and mean fluorescence intensity of monocytes staining positive for IL-6 actually decreases during prolonged running (5). Therefore, the previously held assumption that the IL-6 response to acute exercise may involve immune cells does not appear to be correct. It has been previously shown that physiological and pharmacological stress can lead to increases in the release of

IL-6 from the liver and gut (5). However, the liver appears an unlikely source for the increased circulating IL-6 seen with exercise. We recently had healthy young males exercise for 120 min on a semirecumbent cycle ergometer. By placing catheters in the brachial artery and the hepatic vein, and by measuring blood flow using indocyanine green dye, we were able to quantify IL-6 flux across the hepatosplanchnic viscera. Rather than produce IL-6, the hepatosplanchnic viscera clears IL-6 during exercise, because we observed a net IL-6 uptake by these tissue beds (4). The differences between exercise and other stressors on the capacity for the liver to produce or clear IL-6 are not readily apparent; however, it should be noted that other stressors may result in systemic inflammation, whereas this does not occur with exercise. It must be also noted that small amounts of IL-6 are released from the peritendon and brain but not the adipose tissue during exercise.

In 2000, Steensberg *et al.* (15) published the first article demonstrating that most of the IL-6 seen in the circulation was likely to be derived from the contracting limb. Using a single-legged kicking model and measuring arteriovenous difference and blood flow across the contracting and noncontracting limb, it was clear that net release from the contracting limb was marked. This study was followed by many others that confirmed the net limb release of IL-6 was marked and that the IL-6 mRNA levels in biopsy samples taken from the contracting limb rapidly increased above basal (5). However, it was only recently confirmed that the myocytes *per se* produce IL-6. Using immunohistochemical techniques, Penkowa *et al.* (12) reported a qualitative elevation in IL-6 protein measured in muscle cells within human muscle biopsy sections by immunohistochemistry. These data lent support to the hypothesis that IL-6 is produced in and released from myocytes, but could not categorically confirm because of the possibility that cells other than myocytes produced the IL-6, which then trafficked into the muscle cells. In a follow-up study, however, Hiscock *et al.* (7) provided definitive evidence that myocytes *per se* are a major source of contraction-induced IL-6. In addition to immunohistochemistry techniques, *in situ* hybridization assays were performed on muscle cross-sections before and after exercise. Consistent with the immunohistochemistry data, IL-6 mRNA was almost absent in cross-sections before exercise, but prominent after contraction (Fig. 1).

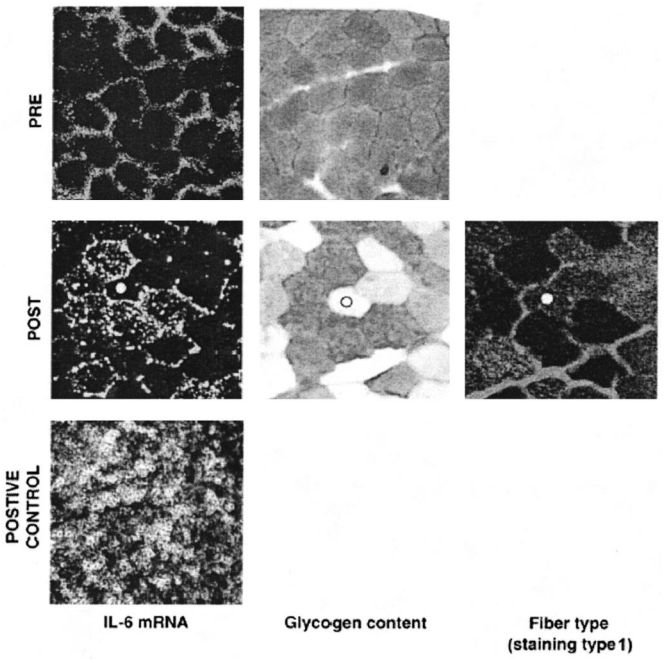

Figure 1. IL-6 mRNA (left images), glycogen content (middle images), and fiber type (type 1 fibers are fluoresced, right image) in muscle biopsy sections before (PRE, upper images) and after (POST, middle images) 120 min of continuous recombinant cycle ergometry. Human metastatic breast tissue was used as a positive control (bottom image). (Reprinted from Hiscock, N., M.H. Chan, T. Bisucci, I.A. Darby, and M.A. Febbraio. Skeletal myocytes are the source of Interleukin-6 mRNA expression and protein release during contraction: evidence of fiber type specificity. *FASEB J.* 18:992–994, 2004. Copyright © 2004 The Federation of American Societies for Experimental Biology. Used with permission.)

WHAT LEADS TO CONTRACTION-INDUCED IL-6 PRODUCTION IN MYOCYTES?

It has been known for some time that skeletal muscle cells are capable of producing IL-6 in response to various chemical stimuli such as incubation with lipopolysaccharide, reactive oxygen species, and inflammatory cytokines. In these circumstances, the upstream signaling events that lead to the induction of IL-6 have been well categorized and the signaling events that lead to IL-6 production in cultured skeletal muscle cells are consistent with experiments conducted in cardiac myocytes and monocytes. Human skeletal muscle appears unique, however, in that it can produce IL-6 during contraction in the absence of observable markers of inflammation (5), linking IL-6 to metabolism rather than inflammation. The factors that lead to IL-6 gene transcription during contraction rather than inflammation are not fully elucidated. However, both intramuscular IL-6 mRNA expression (10) and protein release (14) are exacerbated when intramuscular glycogen is compromised, suggesting that IL-6 is somehow related to glycogen content. Somewhat paradoxically, however, in our recent study (7) both IL-6 mRNA and protein during prolonged (120 min) moderate-intensity (approximately 55% of peak oxygen consumption; $\dot{V}O_2$peak) exercise was almost exclusively expressed in type 2 muscle fibers. Because during this type of exercise type 1 muscle fibers are preferentially recruited, this resulted in an inverse relationship when comparing fiber-specific IL-6 expression and glycogen content (Fig. 1). It must be noted that in studies before this, the relationship between glycogen and IL-6 was observed in mixed muscle biopsy samples; therefore, we interpret these recent data as follows: as glycogen becomes depleted in type 1 fibers during prolonged exercise, type 2 fibers are serially recruited to maintain force. As these fibers are recruited, they transcribe and ultimately produce IL-6.

What factors lead to IL-6 gene transcription? As carbohydrate availability is reduced, the sympathoadrenal response to exercise is exacerbated, and it has been suggested that epinephrine may stimulate IL-6 gene transcription via β-adrenergic stimulation of protein kinase A. There are studies within the literature that show a link between epinephrine concentration and exercise-induced increases in plasma IL-6. However, we recently tested the hypothesis that epinephrine

mediated IL-6 production by skeletal muscle. We incubated rat skeletal muscle *ex vivo* in various concentrations of epinephrine, and measured IL-6 mRNA expression and protein release into the incubation media. Although pharmacological doses (1000 nmol) of epinephrine increased IL-6 mRNA expression, more physiological doses (100 and 10 nmol) had no such effect, whereas epinephrine did not result in IL-6 protein release irrespective of dose (10). We have previously hypothesized that contraction may lead to IL-6 gene transcription via calcium (Ca^{2+}) being released from the lateral sacs of the sarcoplasmic reticulum to activate IL-6 through activation of nuclear factor of activated T cells (8). In preliminary studies, Keller and co-workers were able to show that the calcium inonophone ionomycin increased IL-6 mRNA expression in cultured human myotubes (Keller, Hellsten, Pilegaard, Pedersen, unpublished observations, 2005). Moreover, when muscle strips are incubated with 5 nmol ionomycin, an increase in IL-6 mRNA expression and protein release is observed (8). These observations are consistent with the work by Hiscock *et al.* (7) because type 2 fibers have an approximately 20-fold higher Ca^{2+} release during contraction than do type 1 fibers.

It appears, therefore, that IL-6 gene transcription during "nondamaging" muscle contraction may be related to fiber-type cytosolic Ca^{2+} content and metabolic stress including glycogen availability. To test the possible intracellular signaling events that lead to IL-6 gene transcription, we recently performed a study in which exercise was performed on two occasions, with previous ingestion of a normal or low-carbohydrate diet that reduced preexercise muscle glycogen content (2). Muscle biopsy samples were obtained and analyzed for IL-6 mRNA. In addition, nuclear proteins were isolated from the samples and analyzed for the mitogen activated protein kinases JNK and p38 MAPK. Nuclear fractions were also analyzed for the phosphorylated forms of JNK (p-JNK) and p38 MAPK (p-p38 MAPK) and the abundance nuclear factor of activated T cells and NF$\kappa\beta$. No differences were observed in the protein abundance of total JNK, p38 MAPK, nuclear factor of activated T cells, or NF$\kappa\beta$ before or after exercise when comparing the two trials, but the nuclear abundance of p-p38 MAPK was higher before exercise after the low-carbohydrate diet. As expected, the fold increase in IL-6 mRNA with contraction was potentiated after the low-carbohydrate diet. Interestingly, a highly significant correlation was observed when assessing the preexercise nuclear p-p38 MAPK and contraction-induced fold increase in IL-6 mRNA. To test if this correlation was causally linked, we incubated L6 myotubes in ionomycin with or without the pyridinylimidazole p38 MAPK inhibitor SB203580. Treatments did not affect total nuclear p38 MAPK, but ionomycin increased nuclear p-p38 MAPK and IL-6 mRNA. The addition of SB203580 to ionomycin decreased nuclear p-p38 MAPK and totally abolished the ionomycin-induced increase in IL-6 mRNA (2). The data suggested, therefore, that phosphorylation of p38 MAPK in the nucleus appears to be an upstream target for IL-6, providing new insights into the regulation of IL-6 gene transcription (Fig. 2), although

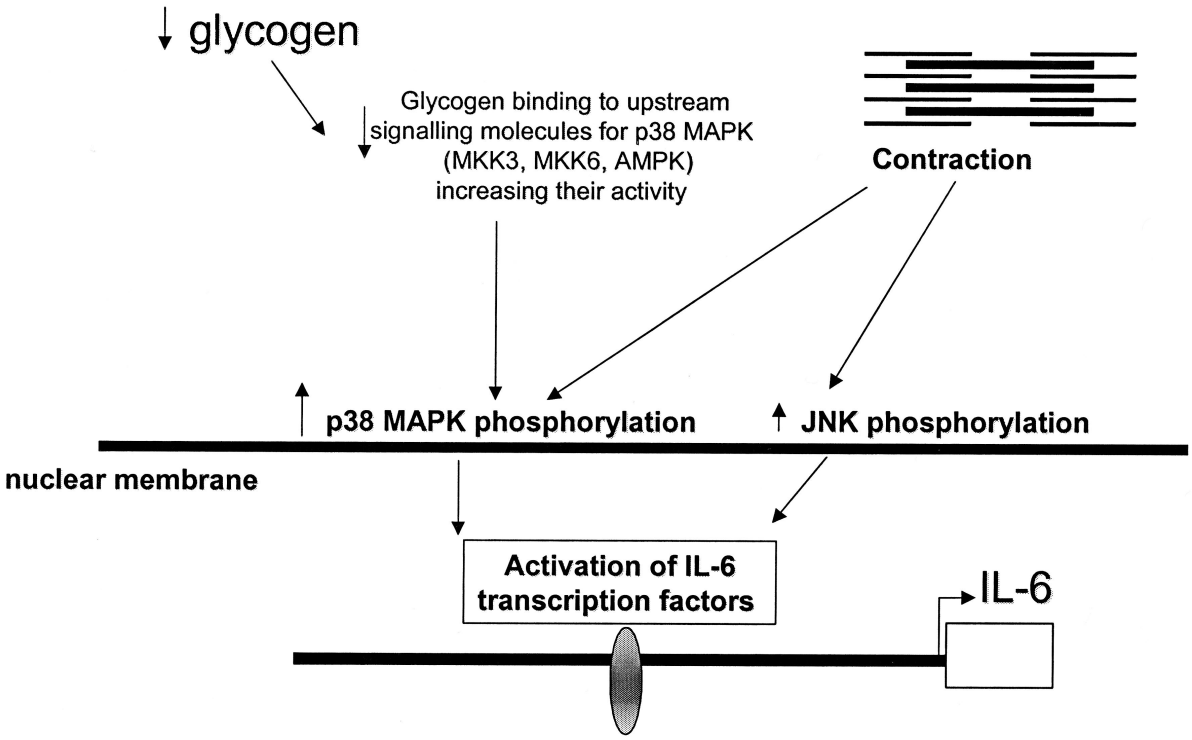

Figure 2. Schematic diagram describing the role of intramuscular glycogen and contraction on the activation of MAPK signaling proteins bound to the nucleus, and the regulation of IL-6 gene transcription. (Reprinted from Chan, M.H.S., S.L. McGee, M.J. Watt, M. Hargreaves, and M.A. Febbraio. Altering dietary nutrient intake that reduces glycogen content leads to phosphorylation of nuclear p38 MAP kinase in human skeletal muscle: association with IL-6 gene transcription during contraction. *FASEB J.* 18:1785–1787, 2004. Copyright © 2004 The Federation of American Societies for Experimental Biology. Used with permission.)

further work is required to determine what binds to the promotor region of the IL-6 gene in myocytes.

WHAT IS THE BIOLOGICAL ROLE OF CONTRACTION-INDUCED IL-6 PRODUCTION AND RELEASE?

Intracellular Downstream Targets

Since the discovery that muscle cells may synthesize IL-6, research has focused on the biological importance of such a phenomenon. In mammalian cells, IL-6 transduces cellular signals through binding with its membrane-bound receptor (IL-6r) or a cleaved soluble version of the membrane-bound receptor (sIL-6r); the bound IL-6/IL-6r can then associate with the membrane-bound glycoprotein gp130. Heterodimers of IL-6, IL-6r, and gp130 then form, and the intracellular signals are initiated. Because heterodimers of sIL-6r bound to IL-6 are able to bind to and activate the gp130 receptor system, cells that have either extremely low or no expression of IL-6r, but express gp130, such as skeletal muscle are often still responsive to cytokine stimulation given the presence of soluble receptors. The cellular effects of IL-6 are induced through signaling through the Jak-STAT pathway, whereby the IL-6r-gp130 heterodimer activates members of the Janus-activated protein kinases (Jak), Jak1, Jak2, and Tyk2. These proteins then phosphorylate and activate the signal transducer and activator of transcription (STAT)-3 in a multitude of cell types. As a result of STAT-3 signaling, cytokines induce transcription of a family of proteins termed the suppressors of cytokine signaling. In hepatic cells, such expression has been found to negatively affect insulin signaling leading to the hypothesis that IL-6 may lead to insulin resistance. There are studies that have shown a link between insulin resistance and IL-6 expression both *in vitro* and *in vivo*, but a discussion as to whether IL-6 enhances or reduces insulin sensitivity is beyond the scope of this discussion and has been covered in a recently published review (1). However, in muscle cells, even though IL-6 increases suppressor of cytokine signaling-3 protein expression through tyrosine phosphorylation of STAT-3 (Carey and Febbraio, unpublished observations, 2005), it paradoxically appears to phosphorylate acute transforming retrovirus thymoma on serine residue 473 (16), which results in the translocation of glucose transporter-4 from intracellular pools to the plasma membrane. Therefore, in muscle cells, IL-6 may activate insulin-signaling molecules, leading to insulin sensitization, although this has not yet been experimentally confirmed.

Recent studies have also suggested that IL-6 may activate the AMP-activated kinase 5'-AMP-activated protein kinase (AMPK). AMPK, the mammalian homologue of yeast sugar nonfermenting protein-1, is a fuel-sensing enzyme that is activated by changes in the energy state of the cell, as well a number of adipokines and hormones, including adiponectin and leptin. Once activated, AMPK can stimulate fatty acid oxidation and glucose uptake. Ruderman and colleagues (11) recently demonstrated that incubating rat *extensor digitorum longus* muscle with IL-6 increased the phosphorylation of AMPK on threonine residue 172, which ultimately leads to AMPK activation (Fig. 3). In the latter study, we demonstrated that both basal and contraction-induced phospho-AMPK was reduced in the skeletal muscles of IL-6 knockout mice. Hence, IL-6 may be produced in skeletal muscle to activate processes associated with glucose uptake and fat oxidation.

Is IL-6 the So-Called Work Factor?

Unlike many proteins that are produced intramyocellularly, IL-6 is markedly and rapidly released into the circulation. Teleologically, therefore, one would expect that IL-6 is a myocellular signal. Recently, studies have demonstrated that IL-6 infusion or incubation results in lipolysis and fat oxidation both in humans *in vivo* and in skeletal muscle *in vitro* (13). This suggested, to us and others (16), that IL-6 may be released from muscle during exercise to increase lipolysis. When lipolysis is pharmacologically blocked during exercise, circulating IL-6 is markedly increased (9), emphasizing the link between IL-6 and lipolysis during exercise. We recently examined this but, contrary to our hypothesis, IL-6 infusion during exercise at 70% $\dot{V}O_2$peak did not increase the rate of lipolysis or fatty acid disappearance in humans (6).

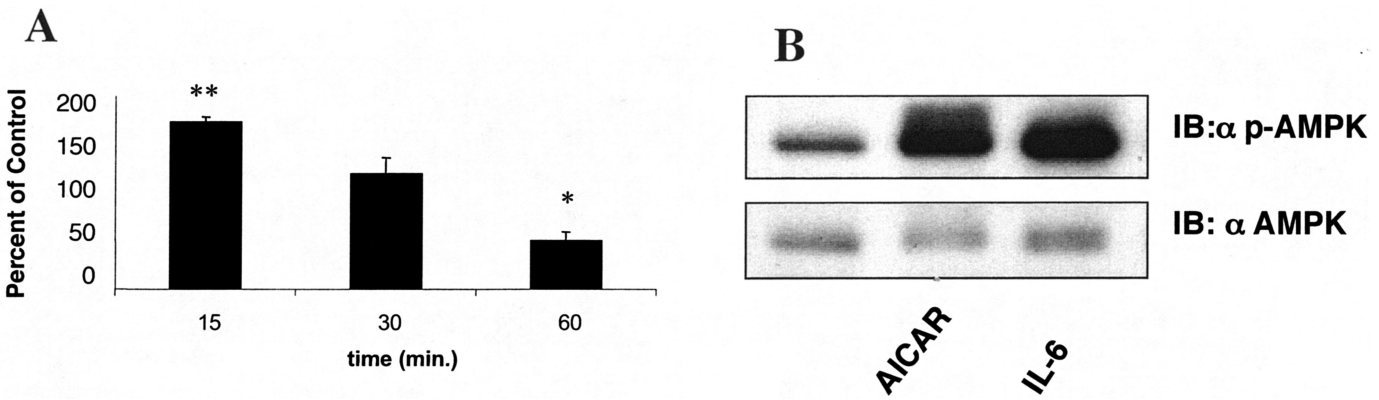

Figure 3. Effect of incubation with IL-6 on the phosphorylation of AMPK. (A) Rat *exterior digitorum longus* muscles (EDL) were incubated with IL-6 (120 ng·mL^{-1}) for indicated times. (B) Cultured F422a adipocytes were incubated with IL-6 (30 ng·mL^{-1}) or AICAR (1 mmol) for 30 min and immunoblotted for both total AMPK and P-AMPK. Results are representative of three blots. (Reprinted from Kelly, M., C. Keller, P.R. Avilucea, P. Keller, Z. Luo, X. Xiang, M. Giralt, J. Hidalgo, A.K. Saha, B.K. Pedersen BK, and N.B. Ruderman. AMPK activity is diminished in tissues of IL-6 knockout mice: the effect of exercise. *Biochem. Biophys. Res. Commun.* 320:449–454, 2004. Copyright © 2004 Elsevier. Used with permission.)

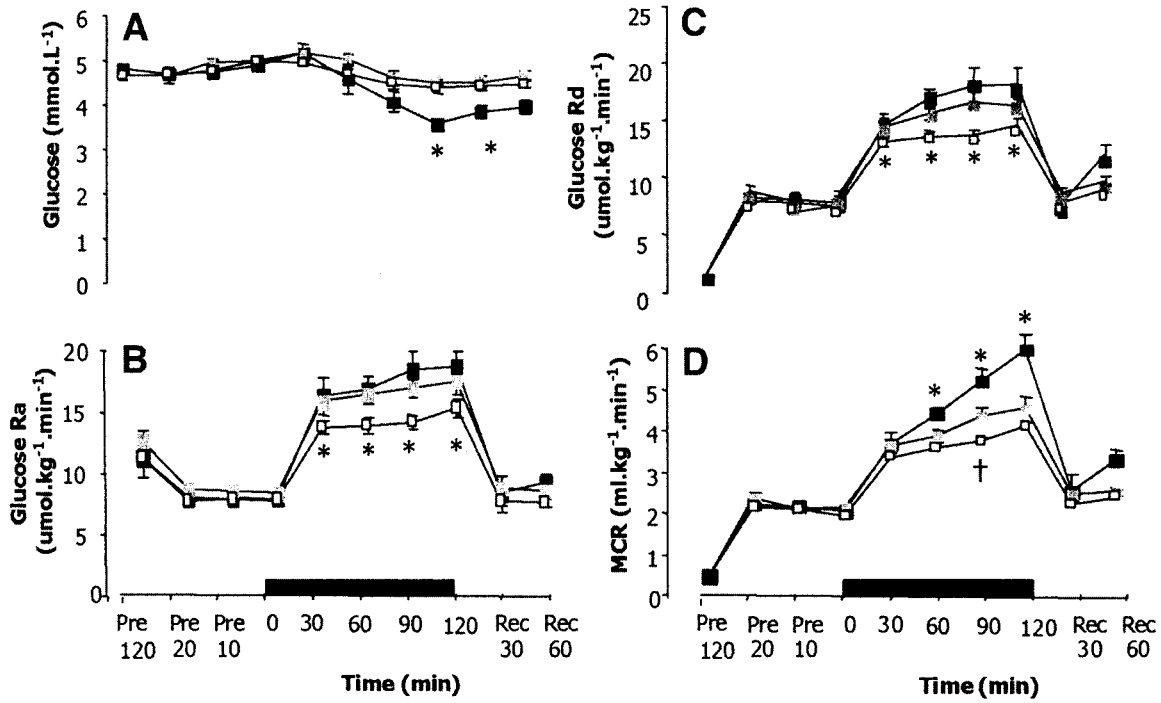

Figure 4. Plasma glucose (A), rate of appearance (Ra) (B), rate of disappearance (Rd) (C), and glucose metabolic clearance rate (MCR) (D) during 120 min of bicycle exercise (and 60 min of recovery) at 70% $\dot{V}o_2$peak (HI, ■) or 40% $\dot{V}o_2$peak without (LO, □) or with (LO + IL-6, ▨) rhIL-6 infusion. *Difference ($P < 0.05$) from HI and LO + IL-6; †Difference ($P < 0.05$) from LO + IL-6. Data are means ± standard error ($N = 6$). (Reprinted from Febbraio, M.A., N. Hiscock, M. Sacchetti, C.P. Fischer, and B.K. Pedersen. Interleukin-6 is a novel factor mediating glucose homeostasis in skeletal muscle contraction. *Diabetes* 53:1643–1648, 2004. Copyright © 2004 American Diabetes Association. Used with permission.)

We concluded that during such exercise other factors that induce lipolysis must be quantitatively more important.

Skeletal muscle contraction is a powerful stimulus for glucose disposal. To maintain glucose homeostasis and avoid hypoglycemia during muscular work, the increase in glucose uptake is accompanied by an increase in endogenous glucose production. Regulation of the contraction-induced increase in endogenous glucose production has been the focus of a vast number of studies over the past 40 yrs. In general, it is accepted that during exercise at a moderate intensity, glucoregulation is primarily mediated by an increase in the portal venous glucagon-to-insulin ratio, but studies have been unable to fully elucidate the precise mediator(s) of contraction-induced endogenous glucose production. As far back as the 1960s, it was suggested that muscle cells possess a "humoral" component, and since this time, many studies have concluded that an as yet unidentified factor released from contracting muscle cells may contribute to the increase in hepatic glucose production. It has been hypothesized by us, and others (16), that IL-6 may be a factor contributing to endogenous glucose production during exercise. We recently tested this hypothesis by having humans perform 2 hr of bicycle exercise on three separate occasions, at a relatively high intensity or at a low intensity, with or without an infusion of recombinant human IL-6 that matched the circulating concentration of IL-6 seen during high-intensity exercise (3). Using stable isotopes, we observed that throughout exercise at the low intensity with IL-6 infusion, glucose appearance and disappearance were higher than exercise at the low intensity without IL-6 (Fig. 4). Moreover, glucoregulatory hormones were identical when comparing these trials. These data suggest that IL-6 influences glucose homeostasis during exercise and provide potential new insights into factors that mediate glucose production and disposal, implicating IL-6 in the so-called work factor.

CONCLUSION

In summary, research over the past 5 yrs has demonstrated that IL-6 is a protein synthesized by muscle cells during contraction to regulate key processes associated with energy transduction. Importantly, research has identified IL-6 as the first "myokine": a cytokine released from muscle cells to modulate metabolic processes in other tissues. Hence, like adipose tissue, skeletal muscle can now be referred to as an endocrine organ. This work opens the door for proteomic research to identify other modulatory myocellular factors that are released during contraction.

Acknowledgments

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