Muscular Interleukin-6 and Its Role as an Energy Sensor

BENTE KLARLUND PEDERSEN

The Centre of Inflammation and Metabolism at the Department of Infectious Diseases, and Copenhagen Muscle Research Centre, Rigshospitalet, the Faculty of Health Sciences, University of Copenhagen, DENMARK

ABSTRACT

PEDERSEN, B. K. Muscular Interleukin-6 and Its Role as an Energy Sensor. *Med. Sci. Sports Exerc.*, Vol. 44, No. 3, pp. 392–396, 2012. During recent years, accumulating data have shown that muscle cells are able to produce and secrete several hundred myokines. The finding that muscles produce and release myokines provides a conceptual basis for understanding some of the molecular mechanisms underlying organ cross talk, including muscle–liver and muscle–fat cross talk. The myokine prototype is interleukin-6 (IL-6). During exercise, contracting skeletal muscles release IL-6. It seems that IL-6 works as an energy sensor and exerts both local and endocrine metabolic effects. Given that the skeletal muscle is the largest organ in the human body, the discovery of contracting muscle as a cytokine-producing organ opens for a whole new paradigm: If the endocrine function of the muscle is not stimulated through contractions, it will cause malfunction of several organs and tissues of the body. **Key Words:** CYTOKINES, INTERLEUKINS, DIABETES, CANCER, DEMENTIA, SKELETAL MUSCLE, ADIPOSE TISSUE

The identification of the skeletal muscle as an endocrine organ provides a conceptual basis to understand and explain how physical exercise may protect against widespread diseases, such as type 2 diabetes and ischemic heart disease (23). Our global hypothesis is that contracting skeletal muscles release myokines, which work in a hormone-like fashion, exerting their effects on other organs. This unconventional hypothesis provides an alternative perception of muscle–organ cross talk and creates a new understanding of how muscles communicate with other organs such as the liver, the pancreas, and the brain (25,27).

MUSCLE AS AN ENDOCRINE ORGAN

In line with the acceptance of adipose tissue as an endocrine organ, we came up with the idea that the skeletal muscle also should be considered an endocrine organ. In the beginning of this millennium, we identified a humoral factor, the cytokine interleukin (IL-6), which was produced and released from contracting muscle cells (34). In continuation, we suggested that cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert paracrine or endocrine effects should be classified as myokines (25,27).

Given that the skeletal muscle is the largest organ in the human body, our discovery of contracting muscle as a cytokine-producing organ opens for a whole new paradigm: Through evolution, muscle has played a central role in orchestrating the metabolism and functions of other organs. This paradigm provides a conceptual basis explaining the multiple consequences of a physically inactive lifestyle. If the endocrine function of the muscle is not stimulated through contractions, it will cause the malfunction of several organs and tissues of the body as well as an increased risk of cardiovascular disease and type 2 diabetes.

MYOKINES IN A HISTORICAL CONTEXT

For most of the last century, researchers sought a link between muscle contraction and changes in peripheral organs in the form of an "exercise factor," which could be released from skeletal muscle during contraction and mediate some of the exercise-induced metabolic changes in other organs such as the liver, adipose tissue, and brain (25,27).

In response to exercise, the dramatically increased glucose uptake by the contracting skeletal muscle corresponds to an

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Address for correspondence: Bente Klarlund Pedersen, M.D., D.MSc., Centre of Inflammation and Metabolism, Rigshospitalet – Section 7641, Blegdamsvej 9, DK-2100, Copenhagen, Denmark; E-mail: bkp@rh.dk.
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increased glucose production by the liver, whereby glucose homeostasis is maintained. How does contracting muscle modulate metabolism in the liver? In response to exercise, adipose tissue increases the release of free fatty acids into the circulation. How do contracting skeletal muscle and adipose tissue communicate? Many individuals claim a stronger feeling of pleasantness after exercise. How do muscles communicate to the brain?

The idea that signaling pathways from contracting muscles to other organs are not solely mediated via the nervous system was supported by the finding that electrical stimulation of paralyzed muscles induced in essentially the same physiological changes in spinal cord–injured patients as in healthy humans (18).

It was obvious that one or more muscle-derived humoral factors existed. For lack of more precise knowledge, it was called the "work stimulus" or the "work factor." In our search for an exercise factor, we found a cytokine, interleukin-6 (IL-6), which is produced by contracting muscles and released into the blood. The identification of skeletal muscle as a cytokine-producing organ soon led to the discovery that muscle-derived cytokines (which we have named myokines) play a role in mediating the exercise-associated metabolic changes, as well as the metabolic changes after training adaptation.

During recent years, increased efforts have focused on elucidating the secretory function of skeletal muscle, which has led to the use of a quantitative proteomics platform to investigate the factors secreted during the differentiation of murine C2C12 skeletal muscle cells. We identified and quantitatively analyzed 635 secreted proteins, including 35 growth factors, 40 cytokines, and 36 metallopeptidases. The latter finding highlighted the important role of skeletal muscle as a secretory organ (11). Although the idea of an "exercise factor" can be traced back many years, the identification of muscle as a myokine-producing organ opens for a whole new field of research.

IL-6: THE MYOKINE PROTOTYPE

Exercise and plasma IL-6. It is well recognized that contracting skeletal muscle may synthesize and release IL-6 into the interstitium as well as into the systemic circulation in response to a bout of exercise (23–25). Although several sources of IL-6 have been demonstrated, contracting muscles contribute to most of the IL-6 present in the circulation in response to exercise. The magnitude of the exercise-induced IL-6 response is dependent on the intensity and especially duration of the exercise, whereas the mode of exercise has little effect (8).

The fact that the plasma concentration of IL-6 increases during exercise has been a consistent finding (25). The increase in IL-6 is followed by the appearance of IL-1 receptor antagonist and the anti-inflammatory cytokine IL-10. Concentrations of the chemokines IL-8 and macrophage inflammatory proteins α and β are elevated after a strenuous exercise. Of note, the cytokine's response to exercise and sepsis differs concerning tumor necrosis factor α (TNF-α). Thus, the cytokine's response to exercise is not preceded by an increase in plasma TNF-α. Although there may be a moderate increase in the systemic concentration of these cytokines, the underlying fact is that the appearance of IL-6 in the circulation is by far the most marked and precedes that of the other cytokines (25).

Exercise-induced plasma IL-6 concentrations increase in an almost exponential manner. The peak IL-6 level is reached at the end of the exercise or shortly thereafter, followed by a rapid decrease toward preexercise levels. The basal plasma IL-6 concentration may increase up to 100-fold after exercise (26). Because IL-6 is a classic inflammatory cytokine, it was first thought that the IL-6 response was related to muscle damage. However, it has become evident that muscle damage is not required to increase plasma IL-6 during exercise. Rather, eccentric exercise may result in a delayed peak and a slower decrease of plasma IL-6 during recovery (25).

Contracting skeletal muscle *per se* is the main source of the IL-6 in the circulation in response to exercise. In resting human skeletal muscle, the IL-6 messenger RNA (mRNA) content is very low. But in response to exercise, an increase of the IL-6 mRNA content is detectable in the contracting skeletal muscle after 30 min of exercise, and up to 100-fold increases of the IL-6 mRNA content may be present at the end of the exercise bout (16,33). By obtaining arterial–femoral venous differences in an exercising leg, we found that exercising limbs release IL-6. In an attempt to determine which cells produce the IL-6, Keller et al. (16) isolated nuclei from muscle biopsies obtained before, during, and after exercise. By using reverse transcription–polymerase chain reaction, it was demonstrated that the nuclear transcription rate for IL-6 increases rapidly and markedly after the onset of exercise. This suggested that a factor associated with contraction increases IL-6 transcriptional rate, probably in the nuclei from myocytes. Further evidence that contracting muscle fibers themselves are a source of IL-6 mRNA and protein has been achieved by analysis of biopsies from the human vastus lateralis using *in situ* hybridization and immunohistochemistry (12).

IL-6 works as an energy sensor. Skeletal muscle cells are capable of producing IL-6 in response to various stimuli such as incubation with lipopolysaccharide and inflammatory cytokines. In these circumstances, the upstream signaling events that lead to the induction of IL-6 have been well categorized. However, human skeletal muscle seems unique, in that it can produce IL-6 during contraction in the absence of any markers of inflammation and in a strict TNF-independent fashion (14). This finding suggests that muscular IL-6 has a role in metabolism rather than in inflammation. It is interesting that both intramuscular IL-6 mRNA expression (15) and protein release (31) are markedly enhanced when intramuscular glycogen is low, suggesting that IL-6 is somehow related to glycogen content and works as an energy

sensor. In addition, many studies show that glucose ingestion during exercise attenuates the exercise-induced increase in plasma IL-6 (25) and totally inhibits the IL-6 release from contracting skeletal muscle in humans (7,25).

Training status, glycogen, and IL-6. Exercise training involves multiple adaptations including increased pre-exercise skeletal muscle glycogen content, enhanced activity of key enzymes involved in the β-oxidation, increased sensitivity of adipose tissue to epinephrine-stimulated lipolysis, and increased oxidation of intramuscular triglycerides, whereby the capacity to oxidize fat is increased. As a consequence, the trained skeletal muscle is less dependent on plasma glucose and muscle glycogen as substrate during exercise (25,30). Several epidemiological studies have reported a negative association between the amount of regular physical activity and the basal plasma IL-6 levels: the more physically active, the lower basal plasma IL-6 (8). High plasma levels of IL-6 are closely associated with physical inactivity and metabolic syndrome. Moreover, basal levels of IL-6 are reduced after training (8). In addition, it seems that the exercise-induced increase of plasma IL-6 and muscular IL-6 mRNA is diminished by training (9). It is worth noting that although plasma-IL-6 seems to be down-regulated by training, the muscular expression of the IL-6 receptor (IL-6R) seems to be up-regulated. In response to exercise training, the basal IL-6R mRNA content in trained skeletal muscle is increased by ~100% (15). It can therefore be speculated that with training adaptation, the down-regulation of IL-6 is partially counteracted by an enhanced expression of IL-6R, whereby the sensitivity to IL-6 is increased (Fig. 1).

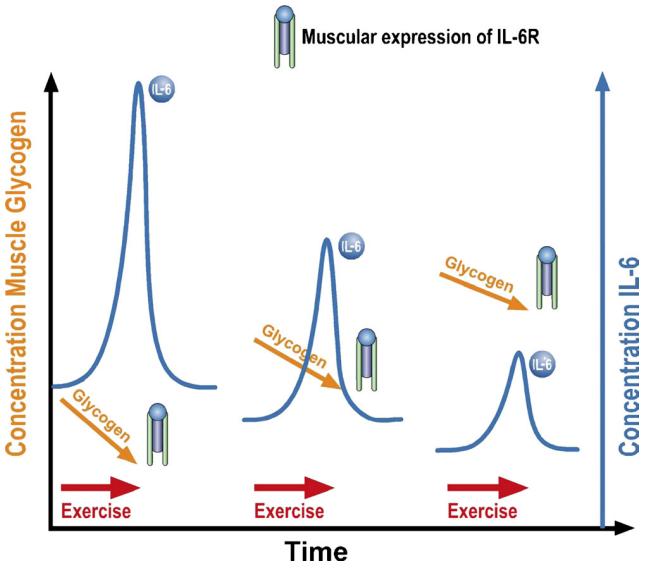

FIGURE 1—Low physical activity is associated with elevated basal IL-6 levels, whereas a high level of physical activity results in low basal IL-6 levels. The finding that differences in training status determines the magnitude of exercise-induced IL-6 responses to exercise is likely due to differences in muscle glycogen content in the trained and untrained skeletal muscle. During acute exercise, the untrained muscle is highly dependent on glycogen as substrate, whereas training leads to an enhancement of β-oxidating enzymes and an enhanced capability to oxidize fat and hence to use fat as a substrate during exercise. This means that the trained muscle uses less glycogen during work. The activation of muscle–IL-6 is glycogen dependent. At conditions with low muscle glycogen, the transcription rate of IL-6 is faster, and relatively more IL-6 is produced at the same relative work compared with conditions with a high muscle glycogen. Thus, the acute plasma IL-6 response is lower in a trained subject versus that in an untrained subject. The mechanisms whereby basal plasma IL-6 is decreased by training and whereby the muscular expression of IL-6Rs is enhanced are not fully understood. However, it seems that a trained muscle may be more sensitive to IL-6. Used with permission from the American Physiological Society 2008, Pedersen and Febbraio (25).

Transcriptional events play a pivotal role in the metabolic adaptations of skeletal muscle. The expression of genes essential for skeletal muscle glucose and lipid metabolism is tightly coordinated in support of a shift in substrate utilization. AMP-activated protein kinase (AMPK) regulates skeletal muscle metabolic gene expression programs in response to changes in the energy status (19). Although AMPK may influence the transcription of metabolic genes, AMPK exerts most of its effects via its role as a protein kinase that regulates the activity of key metabolic enzymes by phosphorylation.

Acute treatment of muscle cells with IL-6 increased both basal glucose uptake and translocation of the glucose transporter GLUT4 from intracellular compartments to the plasma membrane (2). Moreover, IL-6 increased insulin-stimulated glucose uptake *in vitro*, whereas infusion of recombinant human IL-6 into healthy humans during a hyperinsulinemic, euglycemic clamp increased glucose infusion rate without affecting the total suppression of endogenous glucose production (EGP) (2). The effects of IL-6 on glucose uptake *in vitro* seemed to be mediated by the activation of AMPK because the results were abolished in cells infected with an AMPK dominant negative adenovirus (2). Apart from the effects of IL-6 on glucose metabolism, several studies have reported that IL-6 may increase intramyocellular (1,2,29) or whole-body (37) fatty acid oxidation. This effect may, to some extent, be mediated by AMPK (2,13). A recent study suggests that IL-6 activates AMPK in the skeletal muscle by increasing the concentration of cAMP and, secondarily, the AMP/ATP ratio (17). Works from several groups (21,35,39) have demonstrated that leptin may activate AMPK in peripheral tissues such as skeletal muscle. Thus, it seems that IL-6 acutely mediates signaling through the gp130 receptor and exhibits many "leptin-like" actions such as activating AMPK and insulin signaling (36). Although most studies point to an effect of IL-6 on AMPK, Glund et al. (10) provided evidence that AMPK-dependent pathways regulate IL-6 release from isolated oxidative skeletal muscle. It is quite clear that in healthy skeletal muscle, and not least in humans, the IL-6–induced activation of AMPK overrides the IL-6–induced activation of suppressor of cytokine signaling 3. Of note, IL-6 knockout mice develop mature onset obesity and glucose intolerance (38), supporting the notion that IL-6 may exert beneficial effects on metabolism; however, even this observation is unclear (4).

IL-6 is involved in muscle–liver cross talk. The mechanisms that mediate the tightly controlled production and clearance of glucose during muscular work are unclear,

and it has been suggested that an unidentified "work factor" exists that influences the contraction-induced increase in EGP. In healthy humans under the basal condition, acute rhIL-6 (recombinant human IL-6) administration at physiological concentrations does not impair whole-body glucose disposal or a net leg glucose uptake, nor does it increase EGP (20,29, 32). However, we showed that, during exercise, IL-6 contributes to the contraction-induced increase in EGP. Healthy men performed 2 h of bicycle exercise on three separate occasions, at a relatively high intensity (HI) or at a low intensity with (LO + IL-6) or without (LO) an infusion of recombinant human IL-6 that matched the circulating concentration of IL-6 seen in HI exercise. The conclusion from this study was that IL-6 seemed to play a role in EGP during exercise in humans; however, its action on the liver was dependent on a yet unidentified muscle contraction–induced factor (6).

We have recently obtained new perspectives on the muscle–liver axis during exercise (28). The chemokine CXC ligand 1 (CXCL-1) is a small cytokine that elicits effects by signaling through the chemokine receptor CXCR2. CXCL-1 has neutrophil chemoattractant activity; is involved in the processes of angiogenesis, inflammation, and wound healing; and may possess neuroprotective effects. In an experimental study, we found that after a single bout of exercise, CXCL-1 protein increased in serum (2.4-fold) and CXCL-1 mRNA increased in muscle (6.5-fold) and liver (41-fold). These increases in CXCL-1 were preceded by increases in serum IL-6 and muscle IL-6 mRNA. We therefore hypothesized that the myokine IL-6 might mediate the effect of exercise on the liver production of CXCL-1. In support of this idea, we found that exercise-induced regulation of liver CXCL-1 mRNA expression was completely blunted in IL-6 KO mice. Based on these findings, we examined the possible existence of a muscle-to-liver axis by overexpressing IL-6 in muscles. This resulted in increases in serum CXCL-1 (5-fold) and liver CXCL-1 mRNA expression (24-fold) compared with control. Because IL-6 expression and release are known to be augmented during exercise in glycogen-depleted animals, CXCL-1 and IL-6 expression were examined after exercise in overnight-fasted mice. We found that fasting significantly augmented serum CXCL-1 and CXCL-1 expression in liver and muscle (28). These data strongly suggest that Il-6 is involved in a muscle-to-liver communication during exercise.

IL-6 induces lipolysis in skeletal muscle. Infusion of rhIL-6 into healthy humans to obtain physiological concentrations of IL-6 caused an increase in lipolysis in the absence of hypertriglyceridemia or changes in catecholamines, glucagon, insulin, or any adverse effects in healthy individuals (20,29,37). These findings, together with cell culture experiments demonstrating that IL-6 alone increases both lipolysis and fat oxidation, identify IL-6 as a lipolytic factor (29).

In a recent human study, we were able to distinguish between lipolysis in muscle and adipose tissue. We found that rhIL-6 infusion for 4 h increased systemic fatty acid oxidation approximately twofold after 60 min, and it remained elevated even 2 h after the infusion. The increase in oxidation was followed by an increase in systemic lipolysis. In this recent human study, we found that adipose tissue lipolysis and fatty acid kinetics were unchanged with rhIL-6 compared with saline infusion. Conversely, rhIL-6 infusion caused an increase in skeletal muscle unidirectional fatty acid and glycerol release, indicative of an increase in lipolysis. The increased lipolysis in muscle could account for the systemic changes. These findings suggest that an acute increase in IL-6 at a normophysiological level primarily stimulates lipolysis in skeletal muscle, whereas adipose tissue is unaffected (40). The finding that IL-6 has a direct effect on lipolysis is supported by findings from clinical trials. Blocking IL-6 in patients with rheumatoid arthritis leads to enhanced blood lipid and blood glucose levels, indicating that functional lack of IL-6 may lead to insulin resistance and an atherogenic lipid profile (3,5,22). The finding that IL-6 may influence glucose metabolism in peripheral tissues such as muscle and adipose tissue is supported by the finding that IL-6 increases glucose infusion rate and glucose oxidation without affecting the suppression of EGP during a hyperinsulinemic euglycemic clamp in healthy humans (2).

CONCLUSIONS

During recent years, increased efforts have focused on elucidating the secretory function of the skeletal muscle. Through secreted molecules, the skeletal muscle may influence local muscle biology in an auto/paracrine manner while at the same time having systemic effects on other tissues. Muscle-derived IL-6 is the myokine prototype. It is produced by muscle cells and works as an energy sensor. IL-6 seems to have both autocrine/paracrine and endocrine effects. The extensive presence of muscle-derived secreted proteins that can act as potent signaling mediators to other cells and tissues strongly highlights the important role of the skeletal muscle as a prominent secretory organ. The finding that muscles produce and release myokines provides a conceptual basis for understanding some of the molecular mechanisms, whereby muscle communicates to other organs.

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