THE NERVOUS, ENDOCRINE, AND IMMUNE SYSTEMS ALL CONTRIBUTE TO THE MAINTENANCE OF HOMEOSTASIS. THESE SYSTEMS OPERATE INDEPENDENTLY TO A CERTAIN DEGREE, EACH WITH THEIR OWN COLLECTION OF HIGHLY SPECIFIC CELLS AND REGULATORY FACTORS. HOWEVER, THESE SYSTEMS DEPEND ON EACH OTHER FOR NORMAL DEVELOPMENT AND FUNCTION. IN THE EARLY PART OF THE LAST CENTURY, CLASSICAL ABLATION AND REPLACEMENT STUDIES LEADING TO THE PROPOSAL THAT THE ANTERIOR PITUITARY GLAND PLAYS A ROLE IN IMMUNOMODULATION. IN 1930, IT WAS SHOWN THAT REMOVAL OF THE PITUITARY GLAND IN RATS CAUSES THE THYMUS TO INVOLUTE AND ATROPHIES (66).

IN 1976, PELLETIER ET AL. (59) SHOWED THAT IN THE DWARF MOUSE, A MODEL IN WHICH THE PITUITARY FUNCTION IS ABNORMAL, CIRCULATING THYMIC PEPTIDE LEVELS UNDERGO A PREMATURE DECLINE. RESULTS FROM PIONEERING STUDIES SUCH AS THESE LED TO THE HYPOTHESIS THAT NORMAL DEVELOPMENT OF THE IMMUNE SYSTEM IS DEPENDENT ON FACTORS PRODUCED BY THE HYPOTHALAMIC-PITUITARY AXIS. IT WAS SHOWN THAT A NUMBER OF PITUITARY HORMONES (E.G., PROLACTIN, GROWTH HORMONE, AND ACTH) CAN SERVE AS IMMUNOMODULATORY FACTORS (23, 31).

reliance on blood glucose as a source of energy is on the increase” (25).

Our research was originally driven by a curiosity as to whether exercise-induced cytokines would provide a mechanistic explanation to exercise-induced immune changes. However, the identification of skeletal muscle as a cytokine-producing organ soon led to the discovery that not only could muscle-derived cytokines account for exercise-associated immune changes but also that these muscle-derived cytokines played a role in mediating the exercise-associated metabolic changes, as well as the metabolic changes following training adaptation.

For most of the last century, researchers have searched for a muscle contraction-induced humoral factor, an “exercise factor” that could mediate some of the exercise-induced changes in other organs such as the liver and the adipose tissue. We suggested that muscle-derived IL-6 fulfills the criteria of an exercise factor and that such classes of cytokines should be named “myokines” (55). Muscle-derived IL-6 was the first myokine. However, recent research demonstrates that skeletal muscles may produce and express cytokines belonging to distinctly different families. Thus skeletal muscle has the capacity to express cytokines [e.g., IL-6, IL-8, and IL-15 (12, 42)], and muscle contractions play a regulatory role in the muscular expression of these cytokines. Evidence exists that IL-6 (18, 19, 55, 57) and IL-8 (2, 12, 42) are regulated by concentric muscle contractions, both at the mRNA and the protein level, and recently it has been shown that strength training regulates the expression of IL-15 (Nielsen AR, Mounier R, Plomgaard P, Mortensen OH, Penkowa M, Speerschneider T, Damsgaard R, Pilegaard H, Pedersen BK, unpublished data). A bout of exercise provokes the appearance of several cytokines in the circulation, including IL-6, IL-1 receptor antagonist (IL-1ra), IL-8, and IL-10, whereas tumor necrosis factor-α (TNF-α) is only stimulated by very intense exercise (18, 19, 26, 56, 60).

The present review focuses on muscle-derived cytokines, their regulation by exercise, and their possible biological roles and it discusses which cytokines should be classified as a myokine.

THE DEFINITION OF A MYOKINE

We suggest that cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert either paracrine or endocrine effects should be classified as “myokines.”

IL-6

IL-6 belongs to the IL-6 family of cytokines, including IL-11, oncostatin M, leukemia inhibitory factor, ciliary neurotrophic factor, cardiotoxin-1, and cardiotoxin-like cytokine. These cytokines are characterized by their common use of the gp130 [also known as IL-6Rβ (where R is receptor) or CD130] receptor as a signaling subunit. The two IL-6 receptors, gp130 and IL-6Rα (also known as gp80 or CD126), belong to the type I cytokine receptor family, which, in addition to the above cytokines, comprises leptin, growth hormone, prolactin, erythropoietin, thrombopoietin, and granulocyte- and granulocyte/macrophage-colony stimulating factors (33).

The focus of the present review is on muscle-derived IL-6 and its possible local effects in the muscle as well as the possible effects of an acute increase in the systemic levels of IL-6 elicited by a bout of exercise (18, 54, 55). The IL-6 response to exercise has been reviewed (18, 20). In short, a marked increase in circulating levels of IL-6 after prolonged exercise is a remarkably consistent finding. The increase of IL-6 is independent on concomitant muscle damage. The level of circulating IL-6 increases in an exponential fashion (up to 100-fold) in response to exercise, and it declines in the postexercise period (18, 37, 75). The magnitude by which plasma IL-6 increases is related to exercise duration, intensity, the muscle mass involved in the mechanical work, and the endurance capacity. Research within the past years has demonstrated that IL-6 mRNA is upregulated in contracting skeletal muscle (52, 69) and that the transcriptional rate of the IL-6 gene is markedly enhanced by exercise and especially so if muscle glycogen levels are low (34). In addition, it has been demonstrated that the IL-6 protein is expressed in muscle fibers postexercise (30) and that IL-6 is released from skeletal muscle during exercise (72, 73). Exercise was found to increase IL-6 receptor production in human skeletal muscle, suggesting a possible postexercise sensitizing mechanism to IL-6 (35).

IL-6 is most often classified as a proinflammatory cytokine, although data also suggest that IL-6 and IL-6-regulated acute-phase proteins are anti-inflammatory and immunosuppressive and that they may negatively regulate the acute phase response (60, 76). Growing evidence links Type 2 diabetes and cardiovascular diseases to a state of low-grade chronic inflammation, and it has been suggested that IL-6 promotes insulin resistance because of the observation that plasma IL-6 is often elevated in patients with metabolic disease. However, it is now well known that IL-6 is rapidly released into the circulation following exercise (18), and from a simplistic physiological point of view it seems a paradox that working muscle would release a factor that inhibits insulin signaling when insulin action is enhanced in the immediate postexercise period (53). The idea of IL-6 being a “bad or good guy” with regard to metabolic actions has recently been discussed (53). In our view, the finding that IL-6 causes detrimental metabolic effects is at least partly based on correlational relationships in cohort studies together with animal studies and in vitro cell culture studies of supraphysiological concentrations of IL-6. However, it is certainly possible that chronic elevated levels of IL-6 exert proinflammatory effects and have detrimental effects on metabolism.

In response to exercise, IL-6 may be released in significant amounts from the working muscles into the circulation, where it can exert its effect in other organs in a hormone-like fashion. IL-6 is most often classified as a proinflammatory cytokine. However, IL-6 also has anti-inflammatory properties (60). The exercise-induced increase in plasma IL-6 is followed by increased circulating levels of well-known anti-inflammatory cytokines such as IL-1ra and IL-10 (48, 50), and infusion of IL-6 to healthy donors mimics the exercise response on IL-1ra and IL-10 and enhances systemic levels of cortisol (70). Furthermore, both exercise and IL-6 infusion suppress TNF-α production in humans (68). Direct evidence for a role of TNF-α in insulin resistance in humans has been obtained (61), and it is likely that the anti-inflammatory effects of exercise
(mediated by muscle-derived IL-6) may in part protect against TNF-induced insulin resistance.

While IL-6 appears to play a role in endogenous glucose production (EGP) during muscular activity in humans, its action on the liver is totally dependent on other muscle contraction-induced factors (17). At resting conditions, acute IL-6 administration at physiological concentrations does not impair whole body glucose disposal, net leg glucose uptake, or EGP in resting healthy young humans (3, 39, 71). In patients with Type 2 diabetes, plasma insulin decreases in response to IL-6 infusion without a corresponding increase of the hepatic glucose production (3). Recently, our laboratory demonstrated that IL-6 may increase glucose infusion rate (11) and glucose oxidation without changes in EGP during a hyperinsulinemic euglycemic clamp in healthy humans. These data are in contrast with observations reported in mice (53), suggesting that the effects of IL-6 on hepatic insulin sensitivity observed in murine models in vivo may not be similar in humans. The finding of an insulin-sensitizing effect of IL-6 in conditions where EGP was completely suppressed underlines that in humans, the main effect of IL-6 on insulin-stimulated glucose metabolism is likely to occur in peripheral tissues (e.g., skeletal muscle and adipose tissue), whereas IL-6 does not influence glucose output from the liver. Infusion of recombinant human IL-6 into healthy humans to obtain physiological concentrations of IL-6 increases lipolysis in the absence of hypertriglyceridemia or changes in catecholamines, glucagon, insulin or any adverse effects in healthy individuals (3, 39, 77) as well as in patients with Type 2 diabetes (3). These findings, together with cell culture experiments demonstrating that IL-6 alone increases both lipolysis and fat oxidation, identify IL-6 as a novel lipolytic factor. Blocking IL-6 in clinical trials with patients with rheumatoid arthritis leads to enhanced cholesterol and plasma glucose levels, indicating that functional lack of IL-6 may lead to insulin resistance and an atherogenic lipid profile rather than the opposite (14, 45, 46). In accordance, IL-6 knockout mice develop late onset obesity and impaired glucose tolerance (78).

In vivo, experiments demonstrated that IL-6 may increase basal and insulin-stimulated glucose uptake via an increased GLUT4 translocation (11). Recent evidence suggests a link between IL-6 and AMP-activated protein kinase (AMPK). AMPK activation stimulates fatty acid oxidation and increases glucose uptake (32). IL-6 was shown to enhance AMPK activity in both skeletal muscle and adipose tissue (36), and, more recently, the effects of IL-6 on enhanced glucose uptake and fatty acid oxidation in skeletal myotubes were abolished in cells infected with an AMPK dominant negative construct (11).

We find that muscle-derived IL-6 possesses some of the characteristics of a true "exercise factor."

The IL-6 gene is silent in resting muscles, but it is rapidly activated by contractions. The transcription rate is faster than reported for any other gene in muscles, and the fold increase of the transcript may be massive. IL-6 production is modulated by the carbohydrate availability in skeletal muscles, suggesting that IL-6 acts as an "energy sensor." IL-6 released from contracting muscles into the circulation may enhance lipolysis and gene transcription in abdominal subcutaneous fat via its effect on adipose tissue. Muscle-derived IL-6 should be classified as a myokine with endocrine effects.

Furthermore, muscle-derived IL-6 is likely to inhibit low-grade TNF-α production and thereby TNF-α-induced insulin resistance, and it may therefore be a player in mediating the beneficial health effects of exercise.

In summary, IL-6 is expressed by and released from contracting human skeletal muscle, and IL-6 has metabolic effects in humans in vivo. The effect of IL-6 on insulin-stimulated glucose disposal and fatty acid oxidation in humans in vivo appears to be mediated via activation of AMPK.

IL-8

IL-8 was characterized in 1987 by three independent research groups as a neutrophil activating factor. IL-8 belongs to the CXC family of chemokines. The CXC nomenclature relates to the presence of two conserved cysteine residues at the amino terminus separated by one amino acid. IL-8 belongs to a subdivision of CXC-chemokines, which has an amino acid sequence Glu-Leu-Arg (ELR) preceding the first conserved cysteine amino acid residue in the primary structure of these proteins (6). IL-8 is a known chemokine that attracts primarily neutrophils. In addition to its chemokine properties, IL-8 acts as an angiogenic factor.

IL-8, like IL-6, responds to exercise. The plasma concentration of IL-8 increases in response to exhaustive exercise such as running, which involves eccentric muscle contractions (42–44, 51, 74). Concentric exercise on the other hand, such as bicycle ergometry (13) or rowing (29) of moderate intensity, does not increase plasma IL-8 concentration. However, intense cycle ergometry has been reported to increase IL-8 plasma concentration to a small degree (40).

The possibility of contracting skeletal muscle expressing IL-8 has received some attention. In a pioneering study by Nieman and coworkers (42), a severalfold increase in IL-8 mRNA was found in skeletal muscle biopsies from subjects having completed a 3-h-treadmill run concomitant with increased plasma levels of IL-8. Similarly, IL-8 mRNA increased in response to 1 h of cycle ergometry exercise, but there was no change in the plasma concentration of IL-8 (13). In a recent study, our laboratory found that IL-8 protein was clearly expressed in human skeletal muscle as a response to concentric exercise (2). The finding of a marked increase of IL-8 mRNA in muscle biopsies during and following exercise, and of IL-8 protein expression within skeletal muscle fibers in the recovery from exercise, strongly indicates that exercise per se stimulates muscle cells to produce IL-8. This is in accordance with the finding that muscle cells in vitro have the capacity to express IL-8, both at the mRNA and protein levels (15).

The physiological function of IL-8 within the muscle is still unknown. The main part of the systemic increase in IL-8 as seen during exercise with an eccentric component is most likely due to an inflammatory response. In accordance with this, our laboratory and others observed no increase in the systemic IL-8 plasma concentration during or after concentric exercise (2, 12, 29, 42). However, when measuring the arteriovenous concentration difference across a concentrically exercising limb, we detected a small and transient net release of IL-8, which does not result in an increase in the systemic IL-8 plasma concentration (2). That a high local IL-8 expression takes place in working muscle with only a small and transient release could indicate that muscle-derived IL-8 acts locally and...
exerts its effect in an endocrine or paracrine fashion. A plausible function of the muscle-derived IL-8 would be chemotraction of neutrophils and macrophages when, in fact, in concentric exercise there is little or no accumulation of neutrophils or macrophages in skeletal muscle.

A more likely function of muscle-derived IL-8 is to stimulate angiogenesis. IL-8 associates with the CXC receptor 1 and 2 (CXCR1 and CXCR2). It induces its chemotactic effects via CXCR1, whereas CXCR2, which is expressed by human microvascular endothelial cells, is the receptor responsible for IL-8-induced angiogenesis (9, 38, 47). Recently, our laboratory examined the expression of the IL-8 receptor CXCR2 in human skeletal muscle biopsies after concentric exercise (21). Skeletal muscle CXCR2 mRNA increased significantly in response to bicycle exercise in the postexercise period compared with preexercise samples. The increase in CXCR2 protein was seen in the vascular endothelium, and also mildly within the muscle fibers, as determined by immunohistochemistry. This study demonstrated that concentric exercise induces CXCR2 mRNA and protein expression in the vascular endothelial cells of the muscle fibers (21).

In summary, the finding that a high local IL-8 expression takes place in working muscle with only a small and transient release indicates that muscle-derived IL-8 exerts its effect locally. The IL-8 produced by the exercising limb might elicit its response by interacting with the CXCR2 receptor present in the endothelia of capillaries (1, 28). The recent finding that concentric exercise induces CXCR2 mRNA and protein expression in the vascular endothelial cells of the muscle fibers makes us suggest that muscle-derived IL-8 acts locally to stimulate angiogenesis through CXCR2 receptor signaling (21). We suggest that muscle-derived IL-8 should be classified as a potential myokine.

**IL-15**

IL-15 (14-15 kDa) is a four α-helix cytokine with structural similarities to IL-2 (7, 27). Two isoforms of IL-15 with altered glycosylation have been shown to exist: a long signaling peptide form (48 amino acids) that is secreted from the cell, and a short signaling peptide (21 amino acids) form that remains intracellular, localized to nonendoplasmic regions in both cytoplasmic and nuclear compartments. Cell membrane expression might be crucial in mediating an extracellular function rather than secretion and, in part, explains the difficulty in detecting soluble IL-15 in biological systems. IL-15 functions via a widely distributed heterotrimeric receptor (IL-15R), which consists of a β-chain (shared with IL-2) and common γ-chain, together with a unique α-chain (IL-15α) that in turn exists in eight isoforms. Like IL-2, the IL-15Rαβγ complex signals through Janus kinases 1 and 3 and signal transducer and activator of transcription-3 and -5 (8, 24).

The regulatory role of muscle contraction with regard to IL-15 is not clear. Nieman et al. (42) found that muscle IL-15 mRNA levels were not changed immediately after a 3-h run, and Ostrowski et al. (49) found that plasma IL-15 (measured up to 6 h into recovery) did not change in response to 2.5 h of treadmill running. Skeletal muscle IL-15 mRNA levels, measured immediately after a 2-h weight training bout, did not differ from baseline (41), whereas plasma IL-15 protein was increased immediately after acute resistance exercise in one study (65). Our laboratory has recently demonstrated that IL-15 mRNA levels are upregulated in human skeletal muscle following a bout of strength training (Nielsen AR, Mounier R, Plomgaard P, Mortensen OH, Penkowa M, Speerschneider T, Damsgaard R, Pilegaard H, Pedersen BK, unpublished data).

IL-15 has been identified as an anabolic factor, which is highly expressed in skeletal muscle (27). Furthermore, IL-15 has been suggested to play a role in muscle-adipose tissue interaction (5). In human skeletal myogenic cultures, IL-15 induces an increase in accumulation of the protein myosin heavy chain in differentiated muscle cells, suggesting that IL-15 is an anabolic factor in muscle growth (22) and that IL-15 stimulates myogenic differentiation independently of insulin-like growth factors (IGFs) (63). Moreover, in opposition to IGF-I, IL-15 has effects on fully differentiated myoblasts (62). The potential therapeutic effect of IL-15 was demonstrated in an in vivo model, which demonstrated that IL-15 was able to antagonize the enhanced muscle protein breakdown in a cancer cachexia model. Interestingly, while IL-15 has been reliably demonstrated to have anabolic effects on skeletal muscle in vitro and in vivo, IL-15 seems to play a role in reducing adipose tissue mass. When IL-15 was administered to adult rats for 7 days, it resulted in a 33% decrease in white adipose tissue mass (10). The tissue response to IL-15 was related to the amount of IL-15/IL-15 receptor complex expression, suggesting a direct action of IL-15 on adipose tissue (4). IL-15 mRNA expression has been examined in both 3T3-L1 adipogenic cells and C2C12 murine skeletal myogenic cells. Quantitative real-time PCR indicated that IL-15 mRNA was expressed by C2C12 skeletal myogenic cells and that it was upregulated more than 10-fold in differentiated skeletal myotubes compared with undifferentiated myoblasts. In contrast, 3T3-L1 cells expressed little or no IL-15 mRNA on either the undifferentiated preadipocyte or differentiated adipocyte stages (64). These findings provide support for the hypothesis that IL-15 functions in a muscle-to-fat endocrine axis that modulates fat-to-lean body composition and insulin sensitivity.

In summary, IL-15 is a recently discovered anabolic factor that is constitutively expressed by skeletal muscle and regulated by strength training. While IL-15 has solid anabolic effects, it also seems to play a role in reducing adipose tissue mass, and it is therefore suggested that IL-15 may play a role in muscle-fat cross talk. We suggest that muscle-derived IL-15 should be classified as a potential myokine.

**CONCLUSION**

The recent identification of skeletal muscle as an endocrine organ that produces and releases myokines expands our knowledge on how the nervous, endocrine, and immune systems contribute to the maintenance of homeostasis, also when challenged by physiological demands. Given that skeletal muscle is the largest organ in the human body, our discovery of contracting muscle as a cytokine-producing organ opens the possibility for a whole new paradigm: skeletal muscle is an endocrine organ that by contraction stimulates the production and release of cytokines, which can influence metabolism and modify cytokine production in tissues and organs.

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REFERENCES


Invited Review

EXERCISE AND CYTOKINES


