Leukaemia inhibitory factor – an exercise-induced myokine

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ABSTRACT

During and following exercise skeletal muscle synthesises and releases a number of myokines that exert their effects either systemically or locally within the muscle. Several of these myokines influence metabolism, regeneration and/or hypertrophy and are therefore considered to be important contributing factors in muscle homeostasis and muscle adaptation to exercise training. Leukaemia inhibitory factor (LIF) is produced and released from muscle cells in vitro and from intact skeletal muscle in vivo. During exercise, skeletal muscle potently up-regulates LIF mRNA expression, likely due to oscillations in intracellular Ca^{2+} concentrations. However, circulating levels of LIF are not increased with exercise suggesting that LIF exerts its effect locally. LIF stimulates muscle satellite cell proliferation and is involved in muscle hypertrophy and regeneration. Thus, LIF may be produced by skeletal muscle during exercise to contribute to local aspects of muscle adaptation to exercise.

Keywords:
LIF, exercise, skeletal muscle, myokine, satellite cells

INTRODUCTION

During the last decade myokines have been identified as contributing factors in muscle adaptation to exercise (28). Myokines are proteins that are synthesized and secreted by skeletal muscle and affect other organs (29) or contribute to the regulation of muscle growth and metabolism in an autocrine and/or paracrine fashion (28; 33). The production of myokines may increase during or after exercise due to the activation of contraction-induced signalling pathways, e.g. the calcium signalling pathway (7) or due to changes in energy status within the muscle

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fibres (18). To date, the list of identified myokines comprises interleukin (IL)-6 (36), IL-8 (1), IL-15 (25), fibroblast growth factor 21 (16) and brain-derived neurotrophic factor (22). However, the list is rapidly expanding due to the use of advanced techniques such as proteomics and gene electrotransfer in the search for and validation of novel myokines.

Leukaemia inhibitory factor (LIF) is a newly discovered myokine (7). Nevertheless, LIF was identified already in 1988 as a protein secreted from ascites tumour cells (14). The initial observed function of LIF was its ability to induce terminal differentiation of myeloid leukaemic cells (hence its name LIF). Today it is known that LIF has a wide array of actions, including acting as a stimulus for platelet formation, proliferation of haematopoietic cells, bone formation, neural survival and formation, muscle satellite cell proliferation and acute phase production by hepatocytes (23). LIF is a long chain four α-helix bundle cytokine, which is highly glycosylated and may be present with a weight of 38-67 kDa, which can be deglycosylated to ~20 kDa (15; 32). LIF belongs to the IL-6 cytokine superfamily, which consists of structurally and functionally related proteins named neuropoietins (or gp130 cytokines) (13). The neuropoietins exhibit pleiotropy and redundancy in biological activities, and they all share the gp130 receptor component, a common transducer of their receptor complexes (figure 1) (13). The effects

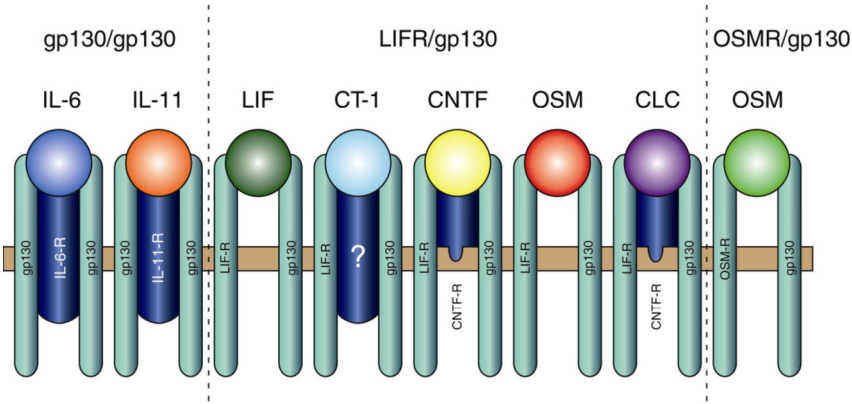

Figure 1. The IL-6 cytokine family and their receptor complexes.
IL-6: interleukin-6, IL-11: interleukin-11, LIF: leukaemia inhibitory factor, CT-1: cardiotrophin-1, CNTF: ciliary neutrophic factor, OSM: oncostatin M, CLC: cardiotrophin-like cytokine, LIFR: leukaemia inhibitory factor receptor.

of LIF are initiated when LIF binds the specific LIF receptor and gp130 (11), which leads to phosphorylation and thereby activation of janus kinase (JAK) and the signal transducer and activator of transcription (STAT)(9; 37). This further results in expression of suppressor of cytokine signalling (SOCS)-3, which negatively regulates LIF signalling at the receptor level (9).

Several tissues, including skeletal muscle, express LIF. Hence, LIF is constitutively expressed at a low level in type 1 muscle fibres (17; 31) and is implicated in

conditions affecting skeletal muscle growth and regeneration (12; 17; 31; 35). LIF protein expression is augmented in mechanically overloaded rat plantaris muscle and in denervated rat muscle (31), thus endogenous LIF production is modulated by factors influencing muscle activity. Furthermore, LIF restores the hypertrophic response to increased loading in LIF (-/-) mice, and in that respect LIF has been denoted as an important factor in skeletal muscle hypertrophy (35). Another, but perhaps related function of LIF is the potency to induce myoblast proliferation and inhibit differentiation of myoblasts into multinucleated myotubes (4; 9; 34; 37). Consequently, LIF seems to affect intact skeletal muscle *in vivo* as well as isolated muscle cell cultures *in vitro*.

LIF is a secreted myokine

Seeing that LIF is produced by skeletal muscle and affects intact muscle as well as isolated muscle cells we hypothesized that LIF would be a myokine. A myokine is defined as a peptide expressed and released by muscle fibres, which exerts autocrine, paracrine or endocrine effects (29). Although the LIF peptide contains a secretory amino acid sequence specifying that LIF be directed out of the cell in which the protein is synthesized (15), no studies have investigated whether LIF is actually secreted from muscle cells or from intact skeletal muscle. We therefore undertook a study to determine the potential of LIF as a secreted myokine. First, we isolated and propagated satellite cells from muscle biopsies obtained from healthy men, as previously described (7), and examined whether the cells could produce and secrete LIF into the cell media. We observed an accumulation of LIF in the cell media (figure 2a), indicating that LIF was produced by cultured muscle cells and secreted spontaneously. Thus, LIF was not stored with-

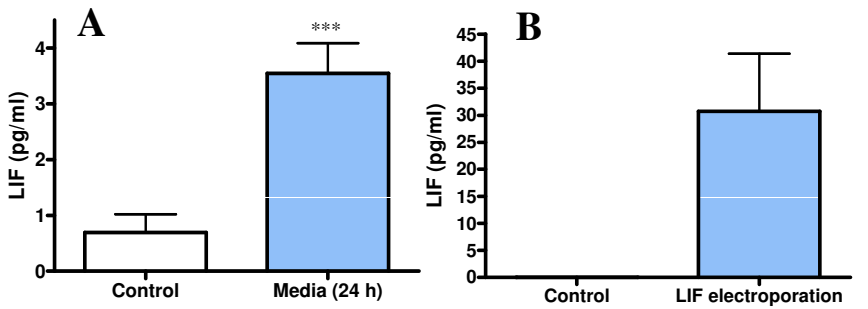

Figure 2. LIF secretion from cultured myotubes and intact skeletal muscle. (A) Satellite cells were isolated from human muscle biopsies and were differentiated into myotubes. The presence of LIF was analyzed by ELISA in fresh differentiation media (control) or in differentiation media covering untreated myotubes for 24 h (*n = 7*).
(B) Mice were electroporated in m. tibialis with a plasmid containing the LIF gene or were given saline injections in m. tibialis (Control). The presence of LIF in plasma was analyzed by ELISA (*n = 8*). Data are expressed as means ± SE.
***Student's *t*-test, p<0.001. LIF: leukaemia inhibitory factor.

in the cells. Secondly, we used the electrotransfer technique, which has previously been described (24), to over express LIF in m. tibialis of mice and assessed the abundance of LIF in serum. Whereas LIF was undetectable in the control mice,

which had saline injected into m. tibialis, the mice electrotransferred with LIF in m. tibialis demonstrated high LIF plasma levels 48 h after the electrotransfer (figure 2b), indicating that LIF was effectively secreted from the intact mouse muscle. Hence, LIF is a muscle-expressed protein released from cultured muscle cells *in vitro* and intact mouse muscle *in vivo*.

LIF is regulated by exercise

In 2008 we demonstrated that aerobic exercise induces expression of LIF in human skeletal muscle (7). Eight male individuals bicycled for 3 h at ~ 60% of VO_2max, and muscle biopsies were obtained before exercise and up to 48 h post exercise. Muscular LIF mRNA expression was ~ 4–fold increased immediately after cessation of exercise and declined gradually throughout the post-exercise period (7). This study showed that aerobic exercise and concentric muscle contractions regulate muscular LIF mRNA expression in humans. With regard to the molecular mechanism responsible for the increase in LIF in relation to exercise, it was shown that human muscle cells that are stimulated with the Ca^{2+} ionophore, ionomycin, increase their expression of both LIF mRNA and protein (7). Thus, oscillations in Ca^{2+} concentration may be the signal conveying neuromuscular activity into changes in the transcription of the LIF gene during muscle contractions. Since the human LIF promoter contains three putative nuclear factor of activated T-cells (NFAT) binding sites (5), the calcineurin-NFAT pathway could represent a possible mechanism for LIF gene activation by Ca^{2+} in myocytes. The myokine and cytokine family member IL-6 is also regulated by Ca^{2+}, possibly through calcineurin (6), thereby suggesting that Ca^{2+} oscillations constitute a common signal to increase transcription of myokines during exercise.

In a recent study, which involved eight healthy male individuals, we found that ~ 20 min of heavy resistance exercise of m. quadriceps induced a ~ 9–fold increase in LIF mRNA in skeletal muscle (Broholm et al., *under revision*). This evidenced that eccentric muscle contractions and resistance exercise regulate LIF. Indeed, resistance exercise induced a higher increase in LIF mRNA, despite shorter exercise duration, than did the bicycle exercise trial described above. This may be explained by the different recruitment pattern of muscle fibre types, the different signalling pathways activated, and/or the relatively larger proportion of eccentric contractions incurred during resistance exercise, which might induce a large extent of muscle damage (10). Especially the latter factor may be important since LIF expression is increased in human skeletal muscle exposed to surgery (30) and in regenerating rodent skeletal muscle (17; 21).

Although muscular LIF mRNA levels appear responsive to different types of exercise, LIF protein levels remain unaltered (7), suggesting that repetitive bouts of exercise are necessary to induce accumulation of LIF protein in skeletal muscle, although the latter suggestion needs to be addressed in long term endurance training studies. In addition, muscle-derived LIF seems to be muscle-specific as LIF was undetectable in plasma in human subjects following bicycle exercise (7) as well as following resistance exercise (Broholm et al., *under revision*). Besides detection limitations, it is possible that LIF is secreted to the interstitial space between muscle fibres and never reaches the circulation. This suggests that LIF

does not function as a systemic myokine, as does for example IL-6 (29), but is more likely to affect skeletal muscle in an autocrine and/or a paracrine fashion.

The role of muscle-derived LIF

In 1991, Austin and co-workers demonstrated that LIF stimulates myoblast proliferation in culture (4), thereby showing that LIF functions as a mitogenic growth factor when added experimentally to muscle precursor cells *in vitro*. To date, different groups have confirmed this finding and shown that LIF induces satellite cell and myoblast proliferation, while preventing premature differentiation, by activating a signalling cascade involving JAK1, STAT1 and STAT3 (2; 9; 34; 37). In line with this, the specific LIF receptor is primarily expressed by satellite cells and not by mature muscle fibres (17). Thus, it seems that LIF has the potential to affect satellite cells rather than mature muscle fibres.

Muscle satellite cells start to form at the late stage of vertebrate embryo development (8). In adult muscle, the satellite cells are quiescent and located beneath the basal lamina and the plasma membrane (38). However, in response to muscle injury or exercise the normally quiescent cells become activated, re-enter the cell cycle and start to proliferate. Later in the process, the cells irreversibly withdraw from the cell cycle and fuse with pre-existing myofibres (8). There is increasing evidence that muscle adaptation and hypertrophy depend on the addition of new myonuclei by way of proliferation and further fusion of satellite cells to the adult muscle fibres (8; 27). Hence, LIF may be involved in muscle adaptation to exercise through its potent effect on muscle satellite cells. Indeed, Spangenburg and co-workers (35) showed that LIF (-/-) mice were unable to enlarge their muscle size in response to increased muscle load. However, the hypertrophic muscle response was restored when the mice were given systemic treatments with LIF. Accordingly, the authors suggested that LIF was an important factor in muscle hypertrophy (35). Muscle regeneration is another process relying on activation and proliferation of satellite cells (8), and in this regard LIF also demonstrates *in vivo* effects. LIF stimulates muscle regeneration in mice suffering from muscle dystrophy (19), and LIF (-/-) mice show reduced muscle regeneration following muscle injury (20), thereby demonstrating that LIF is directly involved in regeneration of skeletal muscle. Thus, the possibility exists that the proliferative effects of LIF on satellite cells are closely linked to the role of LIF in muscle hypertrophy and regeneration.

Depending on the type and duration of exercise, muscle adaptation may involve processes such as muscle growth and muscle regeneration. LIF is produced during exercise and might contribute to muscle adaptation following exercise by stimulating muscle satellite cell proliferation, a process important for muscle hypertrophy and regeneration. In consequence, we hypothesize that the primary function of LIF, as a contraction-induced myokine, is that of a mitogenic growth factor affecting nearby satellite cells in a paracrine fashion (figure 3).

LIF in the context of other myokines

The identification of skeletal muscle as a cytokine-producing organ has led to the discovery of different contraction-induced muscle-derived cytokines e.g. IL-6

(36), IL-8 (1), IL-15 (25) and LIF. Muscle-derived IL-6 was the first myokine to be discovered, and Steensberg et al. (36) showed that acute exercise led to a substantial release of IL-6 from the contracting skeletal muscle into the circulation. Consequently, muscle-derived IL-6 has the ability to affect other organs such as the liver, brain and adipose tissue, as well as skeletal muscle itself. In contrast, muscle-derived LIF, IL-8 and IL-15 do not appear to increase systemically due to a release from skeletal muscle in relation to exercise (7; 26). Hence, these factors may rather work locally. Accordingly, the different myokines have diverse targets and functions. Whereas IL-6 influences metabolism in skeletal muscle, adipose tissue and the liver, as well as regulating satellite cell-mediated hypertrophy (29; 33), IL-8 may affect angiogenesis (26), and IL-15 appears to reduce adipose tissue mass (24). In this context, LIF shares a function with IL-6, namely regulation of satellite cells. This is interesting since these cytokines share the gp130 receptor component of their signalling complexes and show high homology in their tertiary structures (3) (figure 1). However, how the timing and balance between these cytokines influence their effect on satellite cell regulation remains to be examined.

It is evident from the aforementioned studies that muscle-derived substances are important contributing factors in muscle adaptation to exercise. Accordingly, myokines may participate in exercise-associated metabolic changes as well as in growth and regeneration of a previously exercised muscle. However, as numerous different growth factors and cytokines are cooperating to maintain skeletal muscle, the role of myokines in muscle adaptation to exercise is rather complex and may likely depend on the net effects of all global changes.

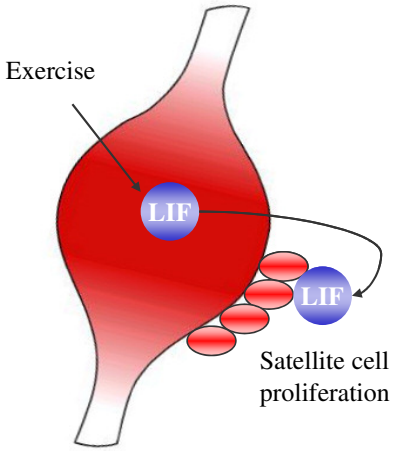

Figure 3. Schematic presentation of the suggested function of muscle-derived LIF. During exercise, skeletal muscle produces and secretes LIF in an autocrine and/or a paracrine fashion. Secreted LIF proteins bind to LIF receptors on satellite cells located near the plasma membrane. This stimulates proliferation of the satellite cells, which participate in muscle adaptation to exercise, e.g. muscle hypertrophy and muscle regeneration. LIF: leukaemia inhibitory factor.

Conclusion and perspectives

The identification of skeletal muscle as an endocrine organ that produces and releases myokines expands our knowledge on how muscle-derived factors contribute to exercise adaptation. Myokines appear to have important local effects within the muscle, including effects on metabolism, angiogenesis and muscle growth. LIF is a newly discovered myokine, which is induced in skeletal muscle following exercise and affects satellite cells, muscle growth and regeneration. The studies on LIF may provide new insight into the complex regulation of muscle tissue maintenance and may

further emphasize the essential role of muscle as an immunogenic organ. We believe that the myokine field will expand in the coming decade and give rise to the identification of new myokines.

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