

# Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword?

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## Note

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Interleukin (IL)-6 is a cytokine with pleiotropic functions in different tissues and organs. Skeletal muscle produces and releases significant levels of IL-6 after prolonged exercise and is therefore considered as a myokine. Muscle is also an important target of the cytokine. IL-6 signaling has been associated with stimulation of hypertrophic muscle growth and myogenesis through regulation of the proliferative capacity of muscle stem cells. Additional beneficial effects of IL-6 include regulation of energy metabolism, which is related to the capacity of actively contracting muscle to synthesize and release IL-6. Paradoxically, deleterious actions for IL-6 have also been proposed, such as promotion of atrophy and muscle wasting. We review the current evidence for these apparently contradictory effects, the mechanisms involved and discuss their possible biological implications.

## Introduction

Adult skeletal muscle is a dynamic tissue capable of responding to environmental stimuli in addition to being an organ that produces and secretes trophic factors. Numerous cytokines and growth factors are produced by the muscle itself, or by infiltrating inflammatory cells during regeneration. These cytokines include hepatocyte growth factor, insulin-like growth factor (IGF1),

fibroblast growth factor, transforming growth factor  $\beta$  and interleukin (IL)-4. These factors have been shown to control skeletal muscle homeostasis, regeneration and growth, in part by regulating critical muscle stem cell (satellite cell) functions. During the last decade, several members of the IL-6 family, particularly IL-6 and leukemia inhibitory factor (LIF), have also been recognized

## Abbreviations

Ang II, angiotensin II; ERK, extracellular signal-regulated kinase; IGF, insulin-like growth factor; IGFBP3, IGF-binding protein-3; IL, interleukin; IL-6R, IL-6 receptor; JAK, Janus kinase; LIF, leukemia inhibitory factor; MAPK, mitogen-activated protein kinase; PIAS, protein inhibitor of activated STAT; SAA, serum amyloid A; SOCS, suppressor of cytokine signaling; STAT, signal transducer and activator of transcription; TGF $\beta$ , transforming growth factor  $\beta$ ; TNF, tumor necrosis factor.

as myokines, that is, cytokines produced by the working skeletal muscle during exercise [1–4].

### The IL-6 family of cytokines

IL-6 was first cloned and characterized in the mid-1980s by several independent groups assessing immunoglobulin production and acute-phase protein responses in different cell lines [5]. Although at first characterized as interferon- $\beta$ 2, amongst other names, it has since gone on to become the cytokine of reference for a subgroup of molecules. IL-6 belongs to the broader four- $\alpha$ -helix family, in which members are structurally defined by a 3D structure of four bundles of  $\alpha$  helices, and is the most studied of the IL-6 family which also includes IL-11, IL-27, IL-31, ciliary neurotrophic factor, cardiotrophin-1, cardiotrophin-like cytokine, LIF, neuropoietin and oncostatin M (reviewed in Ref. [6]). One of the defining features of this family is that they signal via the ubiquitously expressed transmembrane protein gp130 (CD130). Moreover, IL-6 signaling is further complicated by its ability to operate via both 'classical' and 'trans-signaling' mechanisms.

In classical IL-6 signaling, the cytokine first binds to the membrane-bound IL-6 receptor (IL-6R; CD126) that is induced to associate with a homodimer of gp130 which then transmits the intracellular signal. Expression of the IL-6R is limited to several cell types including hepatocytes, neutrophils, monocytes/macrophages and some lymphocytes [7] and thus the range of actions of IL-6 is theoretically limited. However, alternative splicing and limited proteolytic processing of IL-6R generate a soluble and secreted form of the receptor (sIL-6R) that is found in many body fluids. Unlike many other soluble receptors, sIL-6R is not only able to bind to IL-6, but the IL-6–sIL-6 complex is able to bind to and activate gp130 homodimers on cells that do not express membrane-bound IL-6R, thereby mediating 'trans-signaling' and increasing the potential range of IL-6 target tissues and activities [7]. IL-6 family members typically signal through the common gp130 receptor, with the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway being the major intracellular mediator of their effects.

IL-6 is principally defined as a proinflammatory cytokine (reviewed in Ref. [5]). For example, in IL-6-deficient mice, the inflammatory acute-phase response after tissue damage or infection is severely compromised [8]. In addition, IL-6-deficient mice also have reduced IgG and IgA, but not IgM, responses, whereas T-cell activity is also blunted because IL-6 is able to induce the differentiation of B cells to anti-

body-producing plasma cells, and induce T-cell growth and differentiation [5]. IL-6 may also act as a growth factor for some cell types, although it appears to inhibit the growth of others ([5]; see also below), in addition to possessing many other effects. One of its most important roles is in fever because it not only crosses the blood–brain barrier to stimulate prostaglandin E<sub>2</sub> synthesis in the hypothalamus, but it also facilitates peripheral heat production by mediating the mobilization of fuels for heat production in adipose tissue and of course skeletal muscle [9]. Indeed, although IL-6 is by definition pleiotropic, it is also one of the few genuine myokines, or cytokines that are produced by and/or act on skeletal muscle. In response to muscle stress, satellite cells are activated, proliferate, differentiate and fuse to form new myofibers. The IL-6 family of cytokines released by either the muscle compartment (myofibers and/or satellite cells) and/or by infiltrating inflammatory cells might potentially trigger and control the distinct actions of satellite cells throughout the myogenic process.

### Skeletal myogenesis

Formation of skeletal muscle (myogenesis) in the mammalian embryo relies on muscle progenitor cells which express Pax3 and Pax7, two paired-homeobox transcription factors responsible for myogenic lineage specification. After birth, these progenitors adopt a satellite position outside the myofiber and under the basal lamina, hence the name satellite cells. Sometime around postnatal day 21 they enter a quiescent state [10,11]. However, in response to external signals derived from stress or trauma, quiescent satellite cells are activated and undergo asymmetric division to both maintain the satellite cell pool through self-renewal and to generate a progeny of committed myoblasts [12]. It is these myoblasts that subsequently proliferate, migrate, differentiate and fuse into new myofibers, thereby maintaining adult muscle homeostasis and repairing the injured muscle tissue (reviewed in Ref. [11]).

Activation of a muscle-specific transcription factor network composed of four muscle-specific regulatory factors is fundamental for the formation of new myofibers. These muscle-specific regulatory factors, Myf5, MyoD, myogenin and MRF4, all belong to the basic helix–loop–helix family of transcription factors. They cooperate with ubiquitous E proteins (the E2A gene products, E12 and E47, and HEB), and with MEF2 transcription factors on muscle gene promoters, thereby inducing expression of muscle-specific genes [13–16]. Recent studies have also demonstrated an epi-

genetic level of regulation during myogenesis, involving both chromatin modifications and microRNAs [17–19]. Thus, transition of satellite cells from a quiescent (repressive) to an activated state in response to environmental signals is caused by a combination of genetic and epigenetic events, which in turn determine the gene expression program of the satellite cells at the distinct myogenic stages. The IL-6 cytokine family, their downstream effectors and their actions in different models of skeletal muscle growth and repair are the focus of this review.

## **Beneficial effects of IL-6 on muscle formation and growth**

### **Regulation of adult skeletal muscle growth and regeneration by the IL-6 cytokine family**

There are several approaches to studying the role of satellite cells and trophic factors in muscle function. Relatively simpler models such as denervation (atrophy) and overloading (compensatory hypertrophy), where the sciatic nerve is severed or where the soleus and plantaris muscles are forced to compensate for the loss of the surgically isolated gastrocnemius, respectively, are two examples that occur in the absence of inflammation, thereby minimizing the confounding contribution of inflammatory cells. For example, increasing the mechanical load on adult skeletal muscle by overloading constitutes one of the most extreme modes of inducing hypertrophic growth of the tissue. IL-6 and LIF are induced in overloaded muscles during the process of hypertrophy in rodents [20–22]. LIF (and IL-6) expression is also significantly induced by resistance exercise in human muscle and in electrically stimulated cultured human myotubes [3]. Confirming the role of these cytokines in muscle hypertrophy, both LIF and IL-6 knockout mice were shown to have an impaired hypertrophic response to overloading [20,21]. Myofiber hypertrophy in response to overloading requires not only increased net protein synthesis, but also accretion of new nuclei from the progeny of satellite cells. Notably, the impaired hypertrophic muscle growth in IL-6 null mice has been ascribed to blunted accretion of myonuclei, while protein synthesis pathways are preserved [21]. This impaired myonuclei incorporation is a consequence of the defective proliferation and migration capacities of satellite cells in the absence of IL-6 [21], reinforcing the idea that muscle-produced IL-6 critically regulates satellite cell functions. Similarly, LIF has been shown to control the proliferation of satellite cells both in mice and humans [3]. Indeed, exogenous LIF can induce human myo-

blast proliferation, an effect that likely occurs via induction of the cell proliferation-associated factors c-Myc and JunB. These effects can be abrogated by genetic interference with the LIF receptor. Treatment with IL-6 also promotes murine satellite cell proliferation via regulation of the cell-cycle-associated genes *cyclin D1* and *c-myc* [21]. Importantly, a complementary role to IL-6 in stimulating muscle growth may be attributed to IL-4 because it promotes myoblast fusion without affecting their proliferative capacity [23]. Similar to IL-6, IL-4 is produced by exercising muscle [4,24] and the expression of both cytokines has been shown to depend on the transcription factor serum response factor. Thus, serum response factor can be used by the myofibers to translate mechanical cues into paracrine growth-promoting signals that impact positively on satellite cell proliferation and fusion [22].

Functional studies in rodents have shown that IL-6 and LIF also contribute to muscle regeneration after injury and this may involve a similar stimulatory effect on satellite cell proliferation [25–28]. However, the cellular source of IL-6 and LIF in the damaged muscle tissue is less clear than in overloaded muscle. It is likely that distinct cell types contribute to increasing the local production of cytokines, which in turn impact on satellite cells to modify their reparative functions. In regenerating muscle, IL-6 is produced by infiltrating macrophages and neutrophils [28], by fibroadipogenic progenitors [29], as well as by satellite cells [30,31], thus implying potential paracrine and autocrine functions of IL-6 in satellite cell-dependent myogenesis. Early studies showed that cultured human myoblasts produced and secreted IL-6 in response to treatment with proinflammatory cytokines such as IL-1 $\beta$  or tumor necrosis factor (TNF) $\alpha$  [32]. Thus, inflammatory cells infiltrating the injured muscle not only produce IL-6, but also may secrete other proinflammatory cytokines which might lead to further IL-6 expression by satellite cells, thus increasing the concentration of IL-6 in the local satellite cell microenvironment.

This local increase in IL-6 may lead not only to proliferation of satellite cells, but also to their differentiation and fusion, thus playing a dual role in myogenesis. For example, cultured myoblasts undergoing differentiation have been shown to be a source of IL-6 and, more importantly, ablation of IL-6 expression with specific siRNAs reduced the extent of myoblast differentiation and fusion. However, genetic overexpression or addition of exogenous IL-6 augmented the expression of muscle-specific genes, supporting its promyogenic function [33]. The requirement of IL-6 for myogenic differentiation has been recently

confirmed genetically, because myoblasts derived from IL-6 null mice displayed reduced differentiation and fusion capacities *in vitro* [34]. Like IL-6, LIF has also been associated with myoblast differentiation [35]. Thus, the IL-6 family of cytokines appears as a pivotal regulator of myogenesis, acting during both proliferation and differentiation. This dual mode of action is similar to IGF, a critical regulator of myogenesis, which can promote both proliferation and differentiation of satellite cells. Even more interestingly, it has been proposed that during myogenic differentiation, IGF1 can activate the signal transducer and activator of transcription/suppressor of cytokine signaling (STAT/SOCS) pathway [36], which has classically been associated with the intracellular transmission of IL-6 cytokine family signals.

### **Activation of distinct JAK/STAT signaling pathways by the IL-6 family of cytokines regulate satellite cell-dependent myogenesis**

Many intracellular signaling pathways are known to regulate myogenesis. Among them, the p38 mitogen-activated protein kinase (MAPK), insulin-like growth factor/phosphatidylinositol 3-kinase/AKT, calcium/calmodulin-activated protein kinase, and calcineurin positively regulate myogenic differentiation [16,37–40]. Alternatively, the extracellular signal-regulated kinase (ERK) pathway has dual roles: it inhibits differentiation at the early stage of differentiation, but promotes myocyte fusion at the late stages of differentiation [41–43]. Similarly, NF- $\kappa$ B has been shown to promote myoblast proliferation, while also favoring differentiation at later stages by acting as a downstream mediator of p38 MAPK signaling [33,44]. Interestingly, IL-6 expression in differentiating myoblasts was shown to depend on p38 MAPK and NF- $\kappa$ B signaling pathways, and is an effector of their myogenic activities [33].

Consistent with the dual functions of IL-6 and LIF in myogenesis that have emerged in recent years, the JAK/STAT signaling pathway has also been associated with both promotion of myoblast proliferation and/or differentiation. The distinct myogenic actions appear to depend on the particular intracellular mediators of the JAK/STAT pathway engaged at every step. Early studies showed that proliferating satellite cells in regenerating muscle-expressed activated (phosphorylated) STAT3 [31], and cultured myoblasts showed expression of activated STAT3 when stimulated with LIF [45,46]. In addition, STAT3 was shown to be capable of associating directly with MyoD and inhibiting its myogenic activities when overexpressed in C2C12 cells [47].

In recent years, much additional progress has been made in the understanding of the role of the JAK/STAT pathway as an essential intracellular mediator of the IL-6 family of cytokines in myogenesis, particularly from studies by Wu and colleagues [48–51]. *In vivo* analysis of JAK1, STAT1 and STAT3 molecules in regenerating muscles has revealed that they are activated at early times when satellite cells proliferate rapidly [48]. Consistent with this, the JAK1/STAT1/STAT3 pathway was shown to be necessary for myoblast proliferation *in vitro*, based on its capacity to regulate the expression of cell-cycle-associated genes such as *p27*, *p21* and *Id1*. Further analysis showed that stimulation of myoblast proliferation by LIF treatment requires formation of a STAT1/STAT3 complex, because genetic interference with these molecules abrogates LIF-mediated proliferation. This result is consistent with the delayed muscle regeneration of LIF<sup>-/-</sup> mice, which can be rescued by delivery of exogenous LIF [25]. Thus, LIF is essential for the proliferation of myoblasts both *in vivo* and *in vitro*. Consistent with this, IL-6-dependent activation of STAT3 was also shown to be required for satellite cell proliferation in response to muscle overloading, and inhibition of this pathway in IL-6 null mice impaired myofiber hypertrophy *in vivo* as well as satellite cell proliferation *in vitro* [21,22].

However, important studies by Wu and colleagues demonstrated that activation of the JAK1/STAT1/STAT3 pathway not only stimulates myoblast proliferation, but also prevents their premature differentiation by blocking the expression of genes critical for myoblast differentiation and fusion, such as MyoD, MEF2 and myogenin [48]. In this way, the JAK1/STAT1/STAT3 pathway constitutes a differentiation checkpoint, ensuring that differentiation commences only when a sufficient number of myoblast cell progeny have been generated during the proliferative phase. Consistent with this, specific knockdown of JAK1 or STAT1 reduces myoblast proliferation and leads to premature differentiation. Thus, LIF, and to certain extent, IL-6, play dual roles in proliferating myoblasts by inducing the JAK1/STAT1/STAT3 pathway, which is able to promote their proliferation and also inhibit their precocious differentiation. Interestingly, exposure of myoblasts to LIF in differentiating conditions appeared to maintain the number of proliferating cells as differentiation proceeded [52]. More precisely, LIF treatment reduced the percentage of cells positive for active caspase 3 through a MEK/ERK-dependent pathway. Because previous studies had shown that caspase 3 activity is required for myogenic differentiation [53], LIF might inhibit differentiation not only via

modulation of the JAK1/STAT1/STAT3 pathway, but also through inhibition of caspase 3.

At variance with the JAK1-mediated actions, Wu's group demonstrated that JAK2 is required for myogenic differentiation in a pathway requiring STAT2 and STAT3, because pharmacological and genetic interference with JAK2, STAT2 or STAT3 activation prevented the differentiation process [49]. Whereas the JAK1/STAT1/STAT3 pathway repressed the expression of MyoD and MEF2, the JAK2/STAT2/STAT3 pathway enhanced their expression, consistent with their opposite action on myogenesis. The JAK2/STAT2/STAT3 pathway also regulates the expression of hepatocyte growth factor and IGF2 in differentiating cells [49]. The expression of hepatocyte growth factor was shown to be repressed by the JAK2/STAT2/STAT3 pathway at the initial stages of differentiation in agreement with the known role of this growth factor in promoting proliferation and inhibiting differentiation. Alternatively, IGF2 was induced by the same pathway as differentiation progressed, a result consistent with the capacity of IGF2 to stimulate myotube formation and growth [54,55]. The role of STAT2 and STAT3 might not be fully redundant because STAT2 appeared to mediate principally the action of JAK2 on hepatocyte growth factor expression, whereas the expression of IGF2 was mediated by both STAT2 and STAT3 [49]. Taken together, these studies indicate that various members of the JAK/STAT family are involved in the regulation of muscle cell proliferation and differentiation through different partners and effectors, thereby exerting distinct actions throughout myogenesis. It is not yet well known which ligands engage the JAK2/STAT2/STAT3 pathway during myogenic differentiation. Because IL-6 is expressed in differentiating myoblasts, and it promotes their differentiation and fusion [33,34], IL-6 (as well as LIF) [35] may be a potential trigger of these actions.

A conclusion that can be deduced from the studies described above is that the JAK1/STAT1/STAT3 pathway needs to be tuned down for cessation of myoblast proliferation and commencement of differentiation. Consistent with this idea, the kinase activity of JAK1 is reduced upon differentiation [48]. Three families of regulators of JAK/STAT signaling are known: the SOCS family of proteins, the protein inhibitor of activated STAT (PIAS) family of proteins and the SH2-containing phosphatase family of proteins [56]. These proteins target distinct members of the JAK/STAT pathway in distinct cellular compartments. SOCS1 and SOCS3 target JAK1 and gp130, respectively, near the plasma membrane to prevent cytoplasmic STATs from being activated, whereas PIAS1

principally targets activated STAT1 in the cell nucleus and prevents it from binding to DNA. The inhibition at distinct levels and positions might function to ensure that the pathway can be effectively turned off thus allowing progression of myogenesis. The fact that STAT1 and STAT3 are capable of inducing the inhibition of the pathway by activating the expression of SOCS1 and SOCS2, but not PIAS1, in a feedback inhibitory mechanism constitutes a further level of specificity and regulation of this pathway during myogenesis [50]. It is worth noting that in differentiating myoblasts, IGF was shown to induce SOCS gene transcription, suggesting that this protein could be involved in the differentiation process [36]. In agreement with this notion, SOCS3 overexpression in human myoblasts resulted in an increased expression of genes associated with skeletal muscle growth, although the mechanism underlying this effect requires further investigation [36]. Interestingly, SOCS3 signaling during aging appears dysregulated [57,58], and therefore, the decline in the regenerative capacity of muscle with aging may be connected to STAT3/SOCS3 dysregulation. PIAS1 has been shown to modulate myogenesis also through the interaction with proteins that can regulate myoblast differentiation, such as SnoN or Msx1 [59–61]. PIAS1 interacts with and directly sumoylates SnoN, which increases the SnoN capacity to block myogenin gene expression and subsequent cell differentiation [59,61]. In addition, PIAS1 was also shown to interact with and sumoylate Msx1, which is then capable of binding to the MyoD promoter thus repressing MyoD gene expression [60]. However, the precise role of PIAS1 in myogenic differentiation remains debatable, because distinct phenotypes have been obtained after interfering with its expression [59,60]. Therefore, additional studies are necessary to confirm the myogenic function of these molecules. Similarly, further investigation is necessary to decipher the function the SH2-containing phosphatase family inhibitors of JAK/STAT. Although SH2-containing phosphatase has been associated with myoblast differentiation [62], siRNA knockdown did not affect the activity of the JAK1/STAT1/STAT3 pathway during the differentiation process [50].

Finally, evidence that other IL-6 family members oncostatin M and cardiotrophin-1 are also active in myogenesis *in vitro* and *in vivo* was recently provided [51,63]. Oncostatin M was shown to inhibit myoblast differentiation by activating the JAK1/STAT1/STAT3 pathway. STAT1 can interact with, and repress the transcriptional activity of, MEF2 *in vitro*. Furthermore, prolonged expression of oncostatin M in injured skeletal muscles resulted in defective regeneration. By

contrast, treatment of myoblasts with cardiotrophin-1 inhibited their differentiation. However, this action was preferentially mediated through activation of MEK/ERK signaling [51,63], which in turn might interfere with activation of critical myogenic regulatory factors. These findings suggest that cardiotrophin-1 and oncostatin M may be implicated in the maintenance of the undifferentiated state in muscle progenitor cells, in collaboration with the proliferative actions of IL-6 and LIF. Collectively, these data suggest that several members of the IL-6 family of cytokines contribute to myogenesis *in vitro* and muscle regeneration and growth *in vivo*, acting at distinct stages of these processes in a timely and regulated fashion, through distinct signaling pathways and effectors.

### Local and systemic beneficial metabolic effects of muscle-derived IL-6

During the past decade, skeletal muscle has been identified as a secretory organ. In accordance, we have suggested that cytokines and other peptides that are produced, expressed and released by muscle fibers and exert either autocrine, paracrine or endocrine effects should be classified as 'myokines' [4,64,65]. Although myostatin was the first muscle-derived peptide that fulfilled the criteria for a myokine, IL-6 was the first myokine that was found to be secreted into the blood stream in response to muscle contractions [1]. Interestingly, IL-6 was serendipitously discovered as a myokine because of the observation that it increased in an exponential fashion proportional to the length of exercise and the amount of muscle mass engaged in the exercise (for review see Ref. [66]). Circulating levels of IL-6 may increase up to 100-fold, although it should be said that less dramatic increases are more frequent. It is important to highlight the fact that the increase of IL-6 in the circulation occurs during dynamic exercise without any sign of muscle damage [66]. Moreover, the IL-6 response to exercise is not preceded by an increase TNF  $\alpha$ . This finding was in contrast to the common belief that the increase in IL-6 during exercise represented a classic acute-phase response initiated by local damage in the working muscles [67]. It was hypothesized that macrophages were responsible for this increase [68], however, early studies clearly demonstrated that immune cells were not the source of origin of IL-6 [69–71]. It was also made clear that the liver clears, rather than secretes, IL-6 during exercise [72].

Human studies revealed that the nuclear transcription rate for IL-6, as well as the IL-6 mRNA levels are rapidly and markedly increased immediately after

the onset of exercise [73,74]. This finding suggested muscle contraction as such would lead to an increase in IL-6 transcriptional rate within the nuclei from myocytes. Further evidence that contracting muscle fibers themselves were a source of IL-6 mRNA and protein was achieved by analysis of biopsies from the human vastus lateralis using *in situ* hybridization and immunohistochemistry techniques [75]. Other studies applied the microdialysis technique and demonstrated that the concentration of IL-6 within the contracting skeletal muscle may be 5–100-fold higher than the levels found in the circulation and that IL-6 appears to accumulate within the contracting muscle fibers as well as in the interstitium during exercise [76]. The simultaneous measurement of arteriovenous IL-6 concentrations and blood flow across the leg in humans has demonstrated that large amounts of IL-6 are released into the circulation from the exercising leg [77].

Studies on human myoblasts [78,79] and human cultured myotubes [80] have, more recently, added substantial findings to the initial human physiological experiments. It has been shown that IL-6 is locally and transiently produced by growing murine myofibers and associated satellite cells [21]. In addition, IL-6 is released from human primary muscle cell cultures from healthy individuals [81,82] and from patients with type 2 diabetes [82]. Moreover, IL-6 is upregulated in human primary muscle cells following electrical stimulation *in vitro* [83,84].

Skeletal muscle production and release of IL-6 are regulated both by muscle contraction as well as by substrate availability. Thus, when muscle glycogen is low, more IL-6 is produced and released during exercise. This finding is compatible with the idea that muscle-derived IL-6 works as an energy sensor [85,86]. In accordance, enhanced glucose availability and training adaptation attenuate the exercise-sensitive increase in IL-6 plasma concentration [85,87]. The finding that IL-6 is released into the blood stream during exercise and that this release is dependent on substrate availability during exercise, suggested that IL-6 plays a role in maintaining energy status during exercise. To study this hypothesis, recombinant human IL-6 was infused into healthy volunteers and glucose and lipid metabolism were studied using stable isotopes [88–91]. These studies were combined with animal models, studies on isolated muscle as well as cellular studies, and showed that physiologically relevant levels of IL-6 have important acute metabolic effects. One study clearly showed that IL-6 is an endocrine myokine with cardinal biological across organs because it contributes to hepatic glucose production during exercise [92]. It was also discovered that IL-6 enhances fat oxidation in skeletal

muscle via an activation of AMP-activated protein kinase [91,93,94] and enhances lipolysis in skeletal muscle with little effect on adipose tissue [88].

Acute administration of IL-6 enhances glucose uptake and translocation of the glucose transporter GLUT4 from intracellular compartments to the plasma membrane and enhances insulin-stimulated glucose uptake in humans [91]. It has recently been shown that IL-6 transgenic mice with sustained elevated circulating IL-6 display enhanced central leptin action and improved nutrient homeostasis leading to protection from diet-induced obesity [95]. Human studies show that IL-6 mediates anti-inflammatory effects and inhibits lipopolysaccharide-induced increase in circulating TNF [4,64]. Moreover, IL-6 signaling in liver-parenchymal cells suppresses hepatic inflammation and improves systemic insulin action in mice [96]. Thus, IL-6 can induce both pro- and anti-inflammatory effects.

Evidence is emerging that IL-6 is also playing a major role in pancreatic  $\beta$ -cell metabolism and insulin secretion. Bouzakri *et al.* clearly suggested a new route of communication between skeletal muscle and  $\beta$  cells that is modulated by insulin resistance and could contribute to normal  $\beta$ -cell functional mass in healthy subjects, as well as to the decrease seen in type 2 diabetes [97]. In another study, Ellingsgaard *et al.* showed that the exercise-induced glucagon-like peptide-1 (a hormone that induces insulin secretion) response was dependent upon muscle-derived IL-6 [98]. Hence IL-6 mediates cross-talk between insulin-sensitive tissues, the gut and pancreatic islets to adapt to changes in insulin demand by increasing glucagon-like peptide-1 secretion.

Collectively, these data show that IL-6 is produced by contracting skeletal muscle and play important roles in regulating metabolism in other organs. To view skeletal muscle as a secretory organ provides a conceptual basis and a whole new paradigm for understanding how muscles communicate with other organs such as adipose tissue, liver, pancreas, bones and brain, explaining why lack of physical activity appears to be a cause of a whole network of diseases, including cardiovascular diseases, type 2 diabetes, cancer and osteoporosis [65].

## Negative regulation of muscle mass by IL-6

### Is IL-6 signaling directly involved in muscle atrophy?

Since the early 1990s, the implication that elevated systemic IL-6 levels, together with the complexity of

the underlying cytokine network, could participate in the development of the multifactorial syndrome of cachexia have been thoroughly studied [99–102]. Cachexia is often associated with cancer and other pathological conditions and not surprisingly the first experimental evidences for a possible negative role of IL-6 in the control of muscle mass were obtained using animal models of inflammation and tumor induced cachexia. In these models, circulating IL-6 concentrations were elevated, along with those of other inflammatory mediators. Inhibiting IL-6 signaling by neutralizing antibodies was shown to have a protective effect on body weight loss [103,104]. However, whether IL-6 signaling is sufficient and/or has a direct role in the induction of muscle atrophy remains controversial. We focus this section in reviewing the research on the catabolic effects of IL-6 on skeletal muscle and the possible mechanisms involved.

### Effects of IL-6 on muscle protein synthesis and degradation rates

The rate of protein breakdown in isolated rat muscles exposed to recombinant IL-6 [105,106], or after injection of IL-6 into normal mice [107], was found to be unaltered. Similarly, no effect of exogenous IL-6 on the proteolytic rate of rat and murine myotubes could be demonstrated [108], which agrees with the unchanged net muscle protein catabolism in mice after 1 week of daily IL-6 administration [109]. These data are also supported by the lack of ubiquitin gene upregulation in muscle after infusion of a single dose of IL-6 to rats, contrary to the effects observed by treating the animals with inflammatory cytokines like TNF $\alpha$ , interferon- $\gamma$  or IL-1 [110].

In contrast to these data, increased muscle proteolysis was found after administration of high doses or long-term exposure to IL-6 in rats or mice [106,111]. This is the case for transgenic mice engineered to overexpress elevated circulating levels of human IL-6 which display severe muscle atrophy by the age of 10 weeks, together with myofiber-intrinsic activation of the lysosomal enzymes cathepsins B and L and increased proteosomal subunit expression [111,112]. Because blockade of IL-6 signaling by sustained treatment with a mouse IL-6R antibody completely reversed the muscular changes reported in IL-6 transgenic mice [112] and reduced muscle atrophy of tumor-bearing wild-type mice [113], it was proposed that IL-6 might have a permissive role in the development of skeletal muscle degradation and body weight reduction when other circulating cytokines were also present [114,115].

It is important to note that differential effects of IL-6 on muscle have also been reported depending on the dose applied and the manner of administration [116–118]. For example, a significant decrease in myofibrillar protein content was induced in rats by local IL-6 infusion ( $0.7 \text{ pg}\cdot\text{muscle}^{-1}\cdot\text{h}^{-1}$ ) [116]. This approach produced a local IL-6 concentration mimicking the muscle IL-6 concentrations reached after strenuous exercise in humans ( $22 \text{ ng}\cdot\text{L}^{-1}$ ) [119], which did not induce systemic effects on circulating IL-6 levels, nor on body and heart mass. Low systemic doses of IL-6 ( $50 \text{ }\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) infused into rats did not cause a significant reduction of fiber size or muscle weight, or altered contractile properties of the diaphragm muscle [117]. However, much higher doses of IL-6 ( $250 \text{ }\mu\text{g}\cdot\text{kg}^{-1}\cdot\text{day}^{-1}$ ) significantly decreased limb muscle weight by 15% and reduced myofiber size in both fast and slow muscle types, affecting particularly the gastrocnemius muscle. Because this treatment also induced severe cardiovascular alterations, it is not clear to what extent peripheral skeletal muscle atrophy was secondary to myocardial failure and/or any metabolic actions of the cytokine [117].

However, IL-6 may not be required for accelerated muscle protein degradation, even in circumstances in which the cytokine is found at high circulating concentrations. For example, muscles of IL-6 knockout and wild-type mice showed similar increases in total and myofibrillar protein breakdown rates in an experimental model of sepsis, and no increase in muscle protein breakdown rates were found in wild-type mice subjected to a sterile turpentine abscess model that increased IL-6 plasma concentration by 100-fold [107].

In humans, the effects of IL-6 may also depend on the particular context of other systems and organs. In healthy human subjects, infusion of a dose of recombinant IL-6 ( $30 \text{ }\mu\text{g}\cdot\text{h}^{-1}$ ) that mimics the IL-6 plasma concentrations after intense prolonged exercise, slightly increased net muscle protein breakdown [120]. However, the most surprising effects were that this IL-6 administration reduced the concentration of arterial amino acids by 20–40%, despite their net release from the muscle, and that there was an ~50% reduction in total muscle protein turnover. These effects could be ascribed to an increased demand for circulating amino acids by other organs, leading secondarily to their depletion in plasma. In turn, this would lead to a decrease in intracellular synthesis and breakdown of muscle proteins, with a minimal net predominance of protein degradation. Thus, in a healthy state, IL-6 might function as a strong metabolic signal rather than directly controlling protein turnover rates in the muscle [120]. By contrast, in situations in which the

demand for amino acids for acute-phase protein synthesis is elevated, and where the muscle catabolic rates is increased, such as in patients with end-stage renal disease, IL-6 could be released by the muscle independent of amino acid availability. IL-6 would then be related with muscle protein degradation, supporting the notion that IL-6 in this context functions as a prominent protein catabolic signal [121]. In summary, no proatrophic role for IL-6 could be experimentally corroborated in a short time period and/or with low dose treatments. Alternatively, persistent and systemic IL-6 levels may be necessary for the induction of catabolic effects and muscle wasting, with the possible participation of other mediators.

#### **Indirect effects of IL-6 on muscle atrophy: interference with the growth hormone/insulin-like growth factor-1 pathway**

Another layer of complexity of the negative effects of IL-6 on muscle growth is represented by its interference with the growth hormone/insulin-like growth factor-1 (GH/IGF1) axis. Indeed, reduced growth was observed in several transgenic mouse models overexpressing human IL-6 [122]. These mice possess high circulating levels of IL-6 from early stages of life, have normal growth hormone levels, but noticeably reduced serum IGF1 levels and enhanced muscle SOCS3 mRNA expression. Short-term IL-6 administration to wild-type mice and humans also reduced circulating IGF1 levels [122,123]. Similarly, neutralization of IL-6 activities in IL-6 transgenic mice rescued the circulating IGF1 levels and fully restored growth, reinforcing the mechanistic connection between high IL-6 and low IGF1 levels in plasma [124]. IGF1 is produced primarily by the liver and is transported in the blood bound to IGFBP3, the most abundant circulating IGF-binding protein, in heterotrimeric complexes that also contain a glycoprotein termed acid-labile subunit [125]. When carefully examining transgenic animals overexpressing IL-6, which also have reduced circulating IGF1 levels, liver IGF1 production and the amount of functional serum acid-labile subunit were found to be unaffected. However, circulating IGFBP3 was markedly reduced, which was associated with its increased proteolysis [126]. Because a similar reduction of IGFBP3 levels were found in wild-type mice treated with exogenous IL-6, it was concluded that an impairment in the formation of stable ternary complexes of IGF1/IGFBP3/acid-labile subunit negatively affected the half-life of IGF1 in the blood and increased its clearance. The mechanisms by which IL-6 may stimulate IGFBP3 protein degradation have not been clarified.



Importantly, studies in humans revealed that short-term maximal exercise, which is associated with muscle release of IL-6, or acute IL-6 infusion resembling the concentrations of IL-6 in serum observed after exercise, did not affect circulating IGFBP3 levels or its proteolytic degradation rate [123,127,128]. On the contrary, reduced circulating IGF1 and IGFBP3 levels were confirmed in patients with chronically elevated serum IL-6 [122,126]. Overall, these results again support strikingly different actions of acute and persistently circulating IL-6 on the interference with IGF1 levels. IGF1 levels may, in turn, influence muscle IL-6 production. Indeed, sustained IGF1 administration to mice prevented sepsis-induced muscle atrophy and inhibited the muscle IL-6 mRNA upregulation in this model [129]. Finally, an indirect contribution to muscle atrophy by inflammatory cytokines occurring via the hypothalamic–pituitary–adrenal axis should also be considered in chronic conditions. Inflammatory mediators acting on the central nervous system can trigger a catabolic program in skeletal muscle leading to severe atrophy, independent of the actions of the cytokines on the muscle [130]. These effects would be mediated in part by a glucocorticoid response, as muscle atrophy is inhibited in adrenalectomized animals after central nervous system inflammatory stimulus. The contribution of IL-6-induced signaling to this newly described central nervous system-mediated atrophy remains to be fully explored.

### Downstream effectors of IL-6 in muscle atrophy

The specific mediators of IL-6 action in muscle atrophy have not been characterized, but some molecules have been proposed for these proatrophic effects. IL-6 overexpression in transgenic mice [131], or in normal mice by intramuscular electroporation of plasmid DNA [132], decreased muscle mass and induced SOCS3 gene expression, suggesting a putative involvement of SOCS3 in muscle atrophy. In agreement with these findings, infusion of IL-6 in muscle for 2 weeks induced STAT3 activation and SOCS3 transcription, correlating with a local reduction of myofibrillar protein content [116]. SOCS3, in turn, might downregulate IL-6 signaling in this system. Surprisingly, the expression of muscle ubiquitin ligases atrogin-1 and MURF-1 was not affected in the later study, but IL-6 treatment reduced the phosphorylation of ribosomal S6K1 and STAT5 proteins and increased muscle IGF1 expression [116]. In a similar approach, local low doses of IL-6 ( $0.0014 \text{ pg}\cdot\text{mg muscle}^{-1}\cdot\text{h}^{-1}$ ) infused for 2 weeks into rat muscle decreased myofibrillar protein content with respect to the contralateral muscles [118].

These changes were associated with a concomitant reduction of circulating levels of IL-6 and IL-1 $\beta$  and increased local expression of IGF1, SOCS3, atrogin, TNF $\alpha$  and its receptor.

The interpretation of these results with respect to the control of muscle degradation pathways is difficult to make unequivocally. Increased muscle IGF1 levels [133] and STAT3 phosphorylation were also observed in models of hypertrophy [133,134], whereas S6K1 activation appeared dispensable for normal protein synthesis and degradation rates, as well as polysome formation in skeletal muscle [135]. TNF $\alpha$  has been shown to upregulate atrogin-1 expression in skeletal muscle via p38 MAPK-mediated phosphorylation of C/EBP $\beta$  [136–138]. Thus, indirect local upregulation of TNF $\alpha$  and its receptor might have contributed to the reduced myofibrillar protein content after local unilateral IL-6 infusion into rat muscles [118].

Whereas reduced muscle STAT5 levels may indicate local low growth hormone signal activity [139], the direct role for SOCS3 in atrophy is not yet clear, since its expression also increases in paradigms associated with muscle hypertrophy [133] and exercise training [140], and decreases in unloading-induced atrophy models [141]. Augmented SOCS3 expression was reported in experimental cancer-induced muscle wasting in mice undergoing an exacerbated proteolysis leading to a 25% weight loss of limb muscles [142]. Interestingly, in this model, the SOCS3 protein level in cachectic muscles was unchanged or even reduced in the more atrophic muscles versus muscles of control mice despite SOCS3 mRNA upregulation. This finding was interpreted as a JAK/STAT-driven increase in proteolytic degradation of SOCS3, which would in turn lead to persistent STAT3 signaling in the muscle because SOCS3 inhibits STAT3. In this case, STAT3 is the primary mediator of muscle wasting [143].

Indeed, transfection of a plasmid expressing constitutively active STAT3 into normal mouse muscle induced a significant reduction of myofiber cross-sectional area, whereas a dominant negative mutant of STAT3, or electroporation of a STAT3 short hairpin, prevented the atrophy induced by injection of Chinese hamster ovary cells overexpressing IL-6 in athymic nude mice and in the C26 adenocarcinoma mouse model [143]. These data suggest that persistent STAT3 signaling may play an important pathogenic role in the development of muscle wasting in some disease states through yet uncharacterized mechanisms. Interestingly in this regard, a recent study has reported that activation of STAT3 is involved in the regulation of autophagy [144]. The nonreceptor tyrosine kinase Fyn would be activated selectively in the fast-glycolytic

fibers in the fed state, inducing phosphorylation of STAT3 which, in turn, would inhibit expression of the class 3 phosphatidylinositol kinase Vps34 protein, which is necessary for autophagosome formation [144]. Thus, according to this model, persistent STAT3 activation might result in chronic inhibition of macroautophagy which would lead to muscle atrophy [145].

### **Synergy of IL-6 with other molecules and autocrine IL-6 upregulation**

Circulating IL-6 may synergize with other molecules to induce muscular atrophy in some disease states. For instance, activation of the renin–angiotensin system, which is commonly found to be induced in congestive heart failure or chronic kidney disease, is known to cause severe muscle degradation. Indeed, increased muscle proteolysis is reproduced experimentally if angiotensin II (Ang II) is infused into rodents [146]. This Ang II effect, which is associated with low levels of circulating and muscle-intrinsic IGF1, as well as downregulation of active AKT and caspase 3, can be rescued by muscle overexpression of IGF1 [146]. IGF1 stimulation prevents Ang II-induced atrophy by activating AKT and inhibiting Foxo-1-dependent activation of atrogen-1 [147]. Because the Ang II receptor is not expressed by skeletal muscle [138], the proatrophic effects of Ang II must be indirect, and IL-6 has been postulated as a significant mediator. Administration of Ang II to wild-type mice induced elevated plasma IL-6 by release from the liver, whereas Ang II infusion to IL-6-deficient animals did not cause muscle atrophy. Nor did it reduce AKT activity, activated caspase 3, ubiquitin–proteasome-dependent pathways or elevated SOCS3 levels. SOCS3, which is induced by IL-6, has been shown to inhibit insulin-dependent signaling by promoting the proteasomal degradation of insulin receptors [148]. However, IL-6 alone is not sufficient to mediate the atrophic actions of Ang II on muscle tissue. Elevated levels of the acute-phase protein serum amyloid A (SAA), also induced in liver by Ang II, synergistically acts with IL-6 to induce SOCS3 protein expression up to the threshold level required for AKT pathway inhibition. *In vitro* experiments confirmed that neither IL-6 nor SAA alone induce significant changes in protein degradation or myotube size, nor were they able to activate the ubiquitin–proteasome system. However, the combination of IL-6 and SAA induced myotube atrophy and stimulated proteolysis via proteasomal activation. Taken together, these data indicate that Ang II stimulates liver-dependent systemic release of IL-6 and SAA that synergistically target muscle cells to induce muscle proteolysis by

inhibiting IGF1-dependent signaling [138]. In this particular example, an interorgan pathway can be delineated, with liver controlling muscle wasting and IL-6 having a necessary, but insufficient role, because the action of other inflammatory mediators is also required to trigger muscle atrophy.

Hyperactivation of IL-6/STAT3 signaling in muscle may also stimulates the synthesis and systemic release of acute-phase response proteins such as SAA and fibrinogen [142], which may function as an amplifying mechanism for catabolic signals in the muscle, turning muscle into a key player in innate immunity with the capacity of producing fivefold more protein than the liver, considering that skeletal muscle represents ~ 40% of total body weight.

IL-6 signaling in skeletal muscle may induce activation of its own transcription in a sort of positive feedback loop. Autocrine upregulation of IL-6 production is supported by the observation of increased muscle IL-6 mRNA expression after infusion of recombinant IL-6 in humans [149] and in cultured mouse and human myotubes [150]. This regulatory mechanism may involve  $\text{Ca}^{2+}$ -dependent pathways and increased mRNA stabilization [150]. SOCS3 mRNA was significantly increased in rat muscles after a treadmill running training program, together with IL-6 expression and enhanced NF- $\kappa$ B activity [140]. *In vitro* studies using the IL-6 promoter linked to the luciferase reporter gene suggested a positive feedback loop by which exercise-induced IL-6 may activate SOCS3 expression and sustain its own expression through NF- $\kappa$ B-dependent pathways [140], thus suggesting the existence of both positive and negative cross-regulatory loops. This mechanism is consistent with the reported NF- $\kappa$ B activation in rat muscles by exercise [151] and the recent findings in humans showing that NF- $\kappa$ B DNA-binding activity to the IL-6 promoter increases transiently after exercise [152]. Whether similar amplifying mechanisms may be functional in accelerated catabolic conditions deserves further investigation.

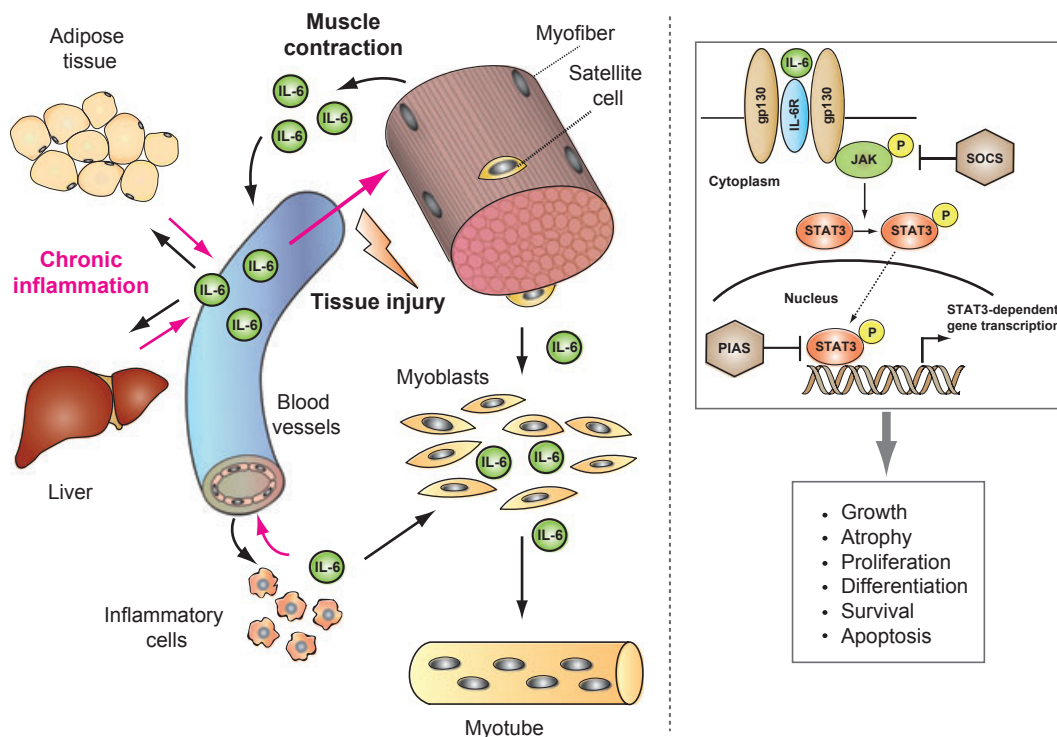
### **Conclusion and perspectives**

Since the discovery of IL-6, many investigations have explored the role of this pleiotropic cytokine and other members of the IL-6 cytokine family on skeletal muscle. It appears consistently in the literature that IL-6, produced locally by different cell types, has a positive impact on the proliferative capacity of muscle stem cells. This physiological mechanism functions to provide enough muscle progenitors in situations that require a high number of these cells, such as during the processes of muscle regeneration and hypertrophic

growth after an acute stimulus. IL-6 is also the founding member of the myokine family of muscle-produced cytokines. Indeed, muscle-produced IL-6 after repeated contractions also has important autocrine and paracrine benefits, acting as a myokine, in regulating energy metabolism, controlling, for example, metabolic functions and stimulating glucose production (see Fig. 1 for an overview of IL-6 production, mechanisms of action and effects). It is important to note that these positive effects of IL-6 and other myokines are normally associated with its transient production and short-term action. The identification of new myokines will potentially serve as novel targets for the treatment of metabolic diseases.

On the contrary, persistent inflammatory conditions and some types of cancer and other chronic disease states are associated with long-lasting elevated systemic

IL-6 levels, which can be reproduced experimentally in different animal model-systems. In such situations, IL-6 actions are coupled with increased muscle wasting, very often acting in combination with other molecules or functioning indirectly to promote atrophy (for example, by inhibiting IGF1-dependent signaling). The direct action of IL-6 as a regulator of atrophy has not been unanimously corroborated by experimental findings. Future studies will take advantage of the available technology that allows the selective interference with IL-6 production, IL-6 receptor and downstream signaling in specific cell types at a desired experimental stage to fully decipher the contribution of the cytokine in different contexts. This knowledge will also potentially allow selective interference of the deleterious actions of IL-6 in pathological contexts and promoting the beneficial effects of IL-6 for therapeutic purposes.



**Fig. 1.** Integrative representation of IL-6 production, signaling mechanisms and local and systemic effects on skeletal muscle and peripheral tissues. (Left) Myofiber released IL-6 after muscle contraction, satellite cell and local inflammatory cell-derived IL-6 after tissue injury impact positively on muscle regeneration and growth by stimulating myoblast proliferation and myotube formation. Muscle-derived IL-6 also has a positive impact on the metabolism of muscle and peripheral tissues, for example, by stimulating glucose availability during exercise and thus acting as an 'energy sensor'. In chronic inflammatory situations, when circulating IL-6 concentration is persistently elevated, a muscle procatabolic effect of the cytokine (pink arrows) has been described by either direct and/or indirect mechanisms (see the text for details). (Right) IL-6-signaling model. The complex of IL-6 and its receptor (IL-6R) binds to the ubiquitously expressed transmembrane protein gp130 homodimer that transmits the signal intracellularly. The major downstream signaling uses the JAK/STAT3 pathway. Once phosphorylated in the cytoplasm, STAT3 migrates to the nucleus and activates transcription on target gene promoters. Members of the SOCS family of proteins and the PIAS family are inhibitors of the JAK/STAT pathway at different levels. The IL-6-induced signaling exerts different effects on distinct cell types and tissues.

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