# Physiological roles of muscle-derived interleukin-6 in response to exercise

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**Purpose of review**

To discuss recent findings with regard to the regulation of muscle-derived interleukin-6 as well as the possible physiological and metabolic roles of interleukin-6 in response to exercise.

**Recent findings**

Contraction-induced transcription and release of interleukin-6 is primarily regulated by an altered intramuscular milieu in response to exercise. Accordingly, changes in calcium homeostasis, impaired glucose availability and increased formation of reactive oxygen species are all associated with exercise and capable of activating transcription factors known to regulate interleukin-6 synthesis. Acute interleukin-6 administration to humans increases lipolysis, fat oxidation and insulin-mediated glucose disposal. Adenosine monophosphate-activated protein kinase activation by interleukin-6 appears to play an important role in modulating some of these metabolic effects. Interleukin-6 facilitates an antiinflammatory milieu and may exert some of its biological effects via inhibition of the proinflammatory cytokine tumor necrosis factor-α.

**Summary**

The discovery of contracting muscle as a cytokine-producing organ opens a new paradigm: skeletal muscle is an endocrine organ that in response to contractions produces and releases 'myokines', which subsequently can modulate the metabolic and immunological response to exercise in several tissues. In our view, interleukin-6 may be one of several myokines.

**Keywords**

adipose tissue, cytokines, insulin resistance, interleukins, skeletal muscle, type 2 diabetes

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**Abbreviations**

| | |
|---|---|
| **AMPK** | adenosine monophosphate-activated protein kinase |
| **IL** | interleukin |
| **MAPK** | mitogen-activated protein kinase |
| **NF** | nuclear factor |
| **rh** | recombinant human |
| **ROS** | reactive organic species |
| **SOCS** | suppressor of cytokine signaling |
| **TNF** | tumor necrosis factor |


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## Introduction

In 2000, it was shown that the active (but not the resting) leg of humans released significant amounts of interleukin (IL)-6 into the circulation during prolonged single-limb exercise [1]. An accompanying editorial by Mike Gleeson said 'It is an intriguing possibility that the IL-6 response may be a signal indicating that muscle glycogen stores are reaching critically low levels and that the active muscles' reliance on blood glucose as a source of energy is on the increase' [2]. Since then, much information has been accumulated with regard to the physiologic and metabolic roles of muscle-derived IL-6. The recent findings with regard to muscle-derived IL-6 will be reviewed.

## Muscle-derived interleukin-6: the first myokine

A marked increase in circulating levels of IL-6 after prolonged exercise without muscle damage is a remarkably consistent finding [3–9]. The level of circulating IL-6 increases in an exponential fashion in response to exercise and declines in the postexercise period [3,4,10].

The magnitude by which plasma-IL-6 increases is related to exercise duration, intensity and muscle mass involved in the mechanical work. Muscle damage is not required in order to increase plasma IL-6 during exercise. Rather, eccentric exercise may result in a delayed peak and a slower decrease of plasma IL-6 during recovery [11,12]. In contrast, the IL-6 response is sensitive to the exercise intensity [13], which again indirectly represents the muscle mass involved in the contractile activity. Since contracting skeletal muscle *per se* is an important source of IL-6 found in the plasma [1,14], it is not surprising that exercise involving a limited muscle mass, e.g. the muscles of the upper extremities, may be insufficient in order to increase plasma IL-6 above pre-exercise levels [15,16]. In contrast, running – which

involves several large muscle groups – is the mode of exercise where the most dramatic plasma IL-6 increases have been observed. The available literature regarding the exercise-induced IL-6 response has been summarized and reviewed recently [17].

Although muscle is a determining factor, the duration of exercise is the single most important factor determining the postexercise plasma IL-6 amplitude. In fact, more than 50% of the variation in plasma IL-6 following exercise can be explained by exercise duration alone. Since exercise at high intensity is associated with shorter duration of the exercise and *vice versa*, the relationship between the plasma IL-6 increase and the duration may be even more pronounced if adjusted for the exercise intensity. Accordingly, 6 min of maximal rowing ergometer exercise may increase plasma IL-6 two-fold [18], but more than 10-fold increases of plasma IL-6 have not been observed in response to exercise lasting less than 1 h. The relationship between duration and the IL-6 response is remarkably insensitive to the mode of exercise, although the highest increases of plasma IL-6 are generally found in response to running. The exercise-induced increase in plasma IL-6 is followed by increased circulating levels of well-known antiinflammatory cytokines such as IL-1ra and IL-10 [13,19].

Within the past few years research has demonstrated that IL-6 messenger RNA is upregulated in contracting skeletal muscle [20,21] and that the transcriptional rate of the IL-6 gene is markedly enhanced by exercise. In addition, it has been demonstrated by immunohistochemistry and in-situ hybridization that the IL-6 protein is expressed in contracting human muscle fibers [22], and that IL-6 is released from working, but not resting, skeletal muscle during exercise [1,23].

In addition, exercise may also increase the IL-6 receptor expression in human skeletal muscle. This increase occurs several hours after cessation of the exercise bout, suggesting a postexercise sensitizing mechanism to IL-6 when the IL-6 levels are declining. Whereas IL-6 receptor protein expression in skeletal muscle is responsive to elevated plasma IL-6 levels, exercise-induced increases in IL-6 receptor messenger RNA most likely occur via an IL-6 independent mechanism as shown in IL-6 knockout mice and from infusion of recombinant human (rh) IL-6 into healthy volunteers [24].

## Training adaptation and interleukin-6

Exercise training involves multiple adaptations including increased skeletal muscle glycogen content, enhanced activity of key enzymes involved in β-oxidation and increased oxidation of intramuscular triglycerides, whereby the capacity to oxidize fat is increased. As a consequence, the trained skeletal muscle is less dependent on plasma glucose and muscle glycogen as substrates during exercise [25]. Epidemiological studies have reported a negative association between the amount of regular physical activity and the basal plasma IL-6 levels: the more physically active, the lower the basal plasma IL-6, reviewed in [17]. Basal plasma IL-6 is more closely associated with physical inactivity than other cytokines associated with the metabolic syndrome [26]. The epidemiological data are supported by findings from intervention studies, although these produce less consistent results. Basal levels of IL-6 are reduced after training in patients with coronary artery disease [27]. Aerobic training of adults aged 64 years or more for 10 months also decreases basal plasma IL-6 [28]. In addition, one study demonstrated that the exercise-induced increase of plasma IL-6 is affected by training. Using knee-extensor exercise, healthy men trained for 1 h, five times a week for 10 weeks [29]. Due to a marked training response, the absolute workload was much higher after training compared to pretraining. Despite this, the increase in IL-6 messenger RNA content by acute exercise at the same relative intensity was 76-fold before training, but only eight-fold after training. In addition, the exercise-induced increase of plasma IL-6 was similar before and after training, although the absolute workload was increased by 44% with training. Accordingly, it is possible that differences in training status explain why elderly subjects release the same amount of IL-6 as young subjects from the leg during knee-extensor exercise at the exact same relative, but half the same absolute, workload [30]. Noteworthy, a training-induced reduction of plasma IL-6 may be partially counteracted by increased expression of IL-6 receptor in the skeletal muscle [31]. The latter finding suggests that a trained muscle is more sensitive to IL-6 than untrained muscle.

## Regulation of muscle-derived interleukin-6

Since IL-6 is synthesized and released only from the contracting muscles and not from the resting muscles exposed to the same hormonal changes [1,32], circulating systemic factors alone do not explain why contracting muscles synthesize and release IL-6. Instead, local factors seem necessary, although systemic factors may modulate the response. The promoter region of the IL-6 gene contains a binding site for the nuclear factor (NF)-κB and NF-IL-6 [33].

Additional transcription factors such as the NF of activated T cells [34] and heat shock factors 1 and 2 [35] may contribute to the activation of IL-6 gene transcription.

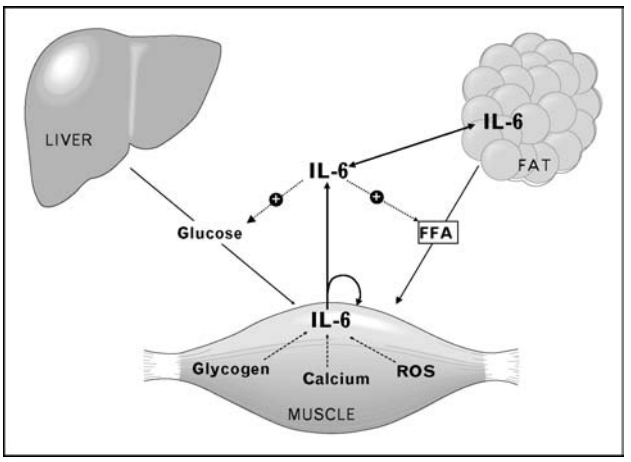
*In vitro*, calcium activates both the NF of activated T cells and NF-κB [36,37], and incubation of muscle cell cultures with a calcium ionophore (ionomycin) increases IL-6 secretion in a p38 mitogen-activated protein kinase

(MAPK)-dependent manner [38,39]. A recent study demonstrates that contraction-induced IL-6 transcription in rat slow-type muscle is partly dependent on calcineurin activation [40]. Human studies have shown increased total and nuclear content of phosphorylated p38 MAPK, but unaltered nuclear content of the NF of activated T cells in muscle biopsies after 1 h of bicycling [41], whilst messenger RNA content of calcineurin A – which is involved in calcium signaling – is increased in muscle biopsies 6 h post 3 h of knee-extensor exercise [42].

Activation of NF-κB has been demonstrated in rat skeletal muscle after exercise [43], but not consistently in humans [41]. Interestingly, NF-κB is a redox-sensitive transcription factor [44] that may be activated by reactive oxygen species (ROS). Increased (ROS) formation in exercising skeletal muscle following exercise has been demonstrated directly in animals [45,46] and indirectly in humans [47]. *In vitro*, murine skeletal myotubes release IL-6 when exposed to oxidative stress in an NF-κB-dependent fashion [48]. In addition, supplementation with different antioxidants attenuates the systemic increase of IL-6 in response to exercise [49,50]. Using arterio-venous differences of IL-6 across the leg, we observed that the reduced systemic increase of IL-6 during exercise was due to an almost complete inhibition of the net leg release of IL-6 in the group pretreated with vitamin C and E for 4 weeks [14]. The observation that indomethacin – a member of the nonsteroidal anti-inflammatory drugs that are known to inhibit NF-κB activity – reduces the exercise-induced increase of IL-6 further supports that NF-κB is likely to serve as a link between contractile activity and IL-6 synthesis [51].

On the other hand, increased oxidative stress, as well as low glucose availability, low glycogen content, catecholamines, increased intracellular calcium levels, hyperthermia and ischemia-reperfusion are all features of exercise capable of inducing heat shock proteins, which may, in turn, activate IL-6 synthesis via heat shock factors 1 and 2 [35]. Accordingly, several regulators of IL-6 transcription are likely to be activated by an altered intramuscular milieu in response to exercise (Fig. 1). This point of view is supported by the various interventions that have demonstrated an effect on the exercise-induced IL-6 response. For instance, reduction of intramuscular glycogen content prior to exercise results increased accumulation of IL-6 messenger RNA within the contracting muscle as well as increased release of IL-6 from the contracting muscle [38,51–53]. This effect of glycogen on the exercise-induced IL-6 may be mediated through activation of p38 MAPK [38] and adenosine monophosphate-activated protein kinase (AMPK) [54]. In contrast, supplementation with carbohydrates during exercise inhibits the exercise-induced increase of IL-6 in plasma, whilst IL-6 messenger RNA expression within the contracting muscle is unaffected [55–58]. Whilst glucose availability may interfere with IL-6 gene expression through AMPK [59,60], other mechanisms regulating IL-6 at a posttranslational level appear to exist. To make it even more complex, IL-6 appears to be capable of enhancing its own transcription [61], which may partly explain the almost exponential increase of IL-6 towards the end of exercise. It should be noted, however, that the IL-6 released into the circulation is cleared very quickly. In mice, the half-life of $^{125}$I-labeled IL-6 in the circulation is 2 min [62], which is in accordance with the fast decrease of plasma IL-6 following rhIL-6 infusion from human studies [63]. A substantial part of muscle-derived IL-6 appears to be cleared by the liver. By placing catheters in the brachial artery and the hepatic vein, and by measuring blood flow using indocyanine green dye, we were able to quantify IL-6 flux across the hepatosplanchnic viscera during exercise. Rather than produce IL-6, the hepatosplanchnic viscera clear IL-6 during exercise, because we observed a net IL-6 uptake by these tissue beds [64].

**Figure 1 Several mechanisms may link muscle contractions to IL-6 synthesis**




Changes in calcium homeostasis, impaired glucose availability and increased formation of ROS are all capable of inducing transcription factors regulating IL-6 gene transcription. The synthesized IL-6 may act locally within the contracting skeletal muscle in a paracrine manner or be released into the circulation, thus able to induce systemic effects. In liver, the circulating IL-6 may increase hepatic glucose output. In adipose tissue, IL-6 produced locally and IL-6 from the circulation in concert may increase lipolysis. FFA, free fatty acids; IL, interleukin; ROS, reactive oxygen species.

## Interleukin-6 and its role in chronic diseases

Growing evidence links type 2 diabetes to a state of low-grade chronic inflammation and it has been suggested that IL-6 promotes insulin resistance due to the observation that plasma IL-6 is often elevated in patients with metabolic disease. From a simplistic physiological point of view, it seems paradoxical that working muscle would release a factor that inhibits insulin signaling when

insulin sensitivity is enhanced in the immediate postexercise period [65]. The idea of IL-6 being a bad or a good guy with regard to metabolism has recently been debated in a counterpoint discussion [66]. Most of the conceptual basis with regard to IL-6 having detrimental metabolic actions is primarily based on (1) correlational relationships in cohort studies, (2) animal studies, neglecting that mouse and human IL-6 exhibit only approximately 42% sequence identity, and (3) in-vitro cell culture studies of supraphysiological concentrations of IL-6.

## The in-vivo effect of interleukin-6 on glucose and lipid metabolism

Using rhIL-6 infusion to humans, we have previously demonstrated that IL-6 appears to play a role in modulating endogenous glucose production during exercise in humans [67]. In contrast, IL-6 has no apparent effects on basal glucose metabolism in resting humans – acute rhIL-6 administration to healthy humans neither impairs whole-body glucose disposal or net leg glucose uptake, nor does it increase endogenous glucose production [68–70]. In fact, in patients with type 2 diabetes, rhIL-6 decreases circulating insulin without concomitant changes in glucose metabolism [70]. To test the hypothesis that IL-6 may increase peripheral insulin sensitivity, we recently demonstrated that IL-6 increases glucose infusion rate and glucose oxidation during a hyperinsulinemic euglycemic clamp in healthy humans [71]. Of note, these data are in contrast to observations reported in mice [72]. The finding of an insulin-sensitizing effect of IL-6 at conditions where endogenous glucose production is suppressed indicates that the main effect of IL-6 on insulin-stimulated glucose metabolism in humans is likely to occur in peripheral tissues such as fat deposits and skeletal muscle.

When infusing rhIL-6 into healthy humans, we found that IL-6 increased lipolysis in the absence of hypertriacylglyceridemia, or changes in catecholamines, glucagon, insulin or any adverse effects. These findings were true both for young and elderly healthy individuals [63,69,70] as well as for patients with type 2 diabetes [70]. Together with cell culture experiments demonstrating that IL-6 alone markedly increases both lipolysis and fat oxidation, these findings identify IL-6 as a novel lipolytic factor [70]. Interestingly, axokine, a human variant of the IL-6 family cytokine member ciliary neurotrophic factor, which acts via a common receptor with IL-6 (the IL-6 receptor/leukemia inhibitory factor receptor/ciliary neurotrophic factor receptor/gp130 receptor complex), induces marked weight loss in obese patients [73]. Moreover, 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 [74–76]. In accordance, IL-6 knockout mice develop late-onset obesity and impaired glucose tolerance [77]. Together, these studies add weight to the notion that IL-6 family cytokines are 'antiobesogenic'.

## Is interleukin-6 acting via adenosine monophosphate-activated protein kinase?

In isolated hepatocytes and in mice *in vivo*, IL-6 has a negative effect on hepatic insulin sensitivity. These findings, however, are in contrast to in-vivo studies in resting humans demonstrating that neither splanchnic glucose output measured by arterio-venous balance across the hepatosplanchnic tissue nor isotopic tracer determined endogenous glucose production is increased by acute infusion of rhIL-6 [68–70]. *In vitro*, IL-6 either enhances [71,78] or does not enhance [79,80] glucose transport in adipocytes. The fact that IL-6 infusion has no effect on subcutaneous adipose tissue glucose uptake in humans [81] suggests that IL-6 has at least no acute effects on insulin-sensitivity in human adipose tissue. In addition, IL-6 increases intramyocellular or whole-body fatty acid oxidation [70] and thus likely to decrease intramyocellular fatty acid accumulation that *per se* may impair insulin signaling. In myocytes, IL-6 may enhance insulin-stimulated glucose transporter 4 translocation, basal and insulin-stimulated glucose uptake [71,82], and glycogen synthesis [83].

Recent evidence suggests a link between IL-6 and AMPK: AMPK activation stimulates fatty acid oxidation and increases glucose uptake [84]. IL-6 enhances AMPK in both skeletal muscle and adipose tissue in mice [85], and the effects of IL-6 on enhanced glucose uptake in skeletal myotubes are abolished in cells infected with an AMPK dominant-negative construct [71]. Studies have shown that IL-6 can enhance lipid oxidation *in vitro* [70], *ex vivo* [86] and *in vivo* [63,70]. AMPK phosphorylates acetyl-CoA carboxylase resulting in inhibition of acetyl-CoA carboxylase activity which, in turn, leads to a decrease in malonyl-CoA content, relieving inhibition of carnitine palmitoyltransferase-1 and increasing fatty acid oxidation [70,84]. We recently showed that the IL-6-mediated phosphorylation of acetyl-CoA carboxylase and subsequent palmitate oxidation *in vitro* is AMPK dependent [71]. These data, together with recent findings regarding ciliary neurotrophic factor, which also enhances lipid oxidation via activation of AMPK in mice [87], suggest that ligands that bind to the gp130 receptor complex generally may enhance glucose uptake and fat oxidation via activation of AMPK.

IL-6 has been shown to activate suppressor of cytokine signaling (SOCS) proteins in liver leading to hepatic insulin resistance [88]. IL-6 increased SOCS3 expression in myotubes, but concomitantly increased glucose uptake in these cells [71]. While IL-6 increased SOCS3 two-fold in muscle, it was increased around 25-fold in liver,

**Table 1 Interventions that modulate the interleukin-6 response to exercise**

| Effect on interleukin-6 in response to exercise | Intervention | References |
|---|---|---|
| Attenuation | Oral carbohydrates supplementation | [55,95–97] |
| | Supplementation with antioxidants | [14,49,50] |
| | Nonsteroidal antiinflammatory drugs (indomethacin) | [98] |
| | Endurance training | [29] |
| Augmentation | Reduction of preexercise of muscle glycogen content | [38,52,53] |
| | Nicotinic acid (reduces lipolysis) | [99] |
| | Heat | [100] |

suggesting that the capacity for IL-6 to induce SOCS3 is much greater in hepatic tissue [89]. Although speculative, the possibility exists that the negative effects of IL-6 on SOCS3 may be overridden by the positive effects on AMPK.

IL-6 stimulates the production of anti-inflammatory cytokines [9] and suppresses tumor necrosis factor (TNF)-α production in humans [90]. Direct evidence for a role of TNF-α in insulin resistance in humans has been obtained [91] and it is likely that muscle-derived IL-6 offers protection against TNF-induced insulin resistance [9]. Given the different biological profiles of TNF-α and IL-6 and given that TNF-α can trigger IL-6 release, it is possible that it is adipose tissue-derived TNF-α that is actually the 'driver' behind the metabolic syndrome and that increased systemic levels of IL-6 reflect locally produced TNF-α [9].

## Skeletal muscle as an endocrine organ

We have known for a long while that the signaling pathways from contracting muscles to other organs were not mediated solely by the nervous system as electrical stimulation of paralyzed muscles in spinal cord injured patients (i.e. lacking afferent and efferent nerve impulses) induces many of the same physiological changes as in intact humans [92,93]. On this basis, it was clear that a humoral factor must exist. For lack of more precise knowledge, such a factor has been called the 'work stimulus' or 'the work factor' [94]. We prefer to use the term 'exercise factor' to cover the effects of muscle contractions as such. In our search for an exercise factor, we found a cytokine, IL-6, which is produced by contracting muscles and released into the blood. We have suggested that muscle-derived IL-6 fulfils the criteria of an exercise factor and that such classes of cytokines should be named 'myokines' [5]. We find that muscle-derived IL-6 possesses some of the characteristics of a true 'exercise factor'. In our view, IL-6 may be one of several 'myokines'. Clearly, the numerous and diverse effects of exercise are not mediated by only or two myokines, but it is possible that there are several myokines that may modulate the more well-known neurohumoral effects. See Table 1.

## Conclusion

Given that skeletal muscle is the largest organ in the human body, the discovery of contracting muscle as a cytokine-producing organ opens a new paradigm: skeletal muscle is an endocrine organ that in response to contractions stimulates the production and release of myokines, which can influence metabolism and modify cytokine production locally and systemically.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 367).


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