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Myokines: Do they really exist?

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Abstract Skeletal muscle has only recently been considered a secretory organ. Muscle-derived proteins are now termed myokines. Until date, about 20 proteins known as cytokines, growth factors, and adipokines have been reported as myokines. However, only a few studies have been able to demonstrate secretion from the skeletal muscle. Furthermore, many reports are still uncertain of whether proteins are secreted from skeletal muscle cells or from the surrounding tissue, because some studies have measured myokine concentration in blood taken from human and animal subjects, which also contains other organ-derived proteins. Secretion of some myokines is promoted by muscle contraction or insulin stimulation, whereas others seem to be constitutively secreted. The mechanisms of action and roles of myokines are also complicated. Some are believed to affect distant organs through endocrine and paracrine mechanisms, while others affect organs through an autocrine mechanism. In this article, we review updates of myokines, including their history. Furthermore, the article discusses the need to re-define myokines in order to avoid possible misunderstandings because of insufficient data.
Keywords : myokine, skeletal muscle, secretion, contraction, exercise

History of myokines

The study of myokines already has a bit of history. About 50 years ago, Goldstein predicted the existence of a humoral factor derived from skeletal muscle that regulates glucose metabolism during exercise[1]. In the early 1990s, the levels of some cytokines were found to be elevated in blood plasma after exercise. An increase in the levels of some cytokines, including interleukin-6 (IL-6), after exercise was considered to originate from immune cells that accumulated in damaged muscle tissue, because most reports focused on cytokines after strenuous exercise[2]. However, other studies in the late 1990s showed that exercise increased plasma IL-6 levels without muscle damage[2-6]. Subsequently, it was thought that some humoral factors might be released from skeletal muscle cells, and these were called myokines (*myo*- means "muscle" and -*kine* means "movement," this terminology comes from "cytokine" in which *cyto*- means cells and -*kine* means movement in Greek). IL-6 has been intensively studied as a myokine. Recently, not only IL-6, but also some humoral factors already known as cytokines, such as IL-8, IL-15, brain-derived neurotrophic factor (BDNF), and fibroblast growth factor-21 (FGF-21)[7,8], have been considered myokines. In addition, recent proteomic analysis indicated that a few hundred proteins are potential myokines[9-11]. These accumulating data led to the gradual wide acceptance of the myokine concept. There is no doubt that some proteins are actually released from skeletal muscle and these have some functions. Therefore, skeletal muscle can be considered a secretory organ, which has brought about new insight into the biological functions of skeletal muscle. However, caution should be exercised when reviewing myokine studies because only a few have provided evidence that molecules are definitively released from skeletal muscle cells and that they can be distinguished from the molecules originating from other tissues/cells surrounding the skeletal muscle.

Definition of myokines

Considering the history of myokines, it may be correct to say that myokines are proteins secreted from skeletal muscles by muscle contraction. However, it is also true that a few hundred proteins are detected in the medium of noncontracted skeletal muscle cells and insulin-stimulated cells[9-11]. Therefore, the definition of myokines needs to be expanded. Pedersen suggested that cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert either paracrine or endocrine effects are classified as myokines[7]. To define myokines, the concept of adipokines, which are released from adipose tissue, may provide an idea. Some descriptions have misused the term "adipokine" to define a protein released by adipose tissue, which also includes nonadipocyte cells such as macrophages, stromovascular cells, or connec-

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tive tissue. Research on myokines is now facing a similar situation. A recent review suggested that "adipokine" refers to any protein secreted from all forms of adipocytes, including white, brown, and brite intermediate types[12]. Although their definition was limited to proteins, another study on adipocyte secretomes stated that adipokines include lipids, proteins, and lactate[13].Therefore, we should carefully review the definition of myokines while considering the definition of an adipokine. It seems that myokines are not necessarily limited to proteins. If analytical techniques are improved in the future, very low-molecular-weight molecules such as lipids and steroids could also become possible candidate myokines. As described above, only one study has defined myokines. Thus, the following points should be considered more extensively: whether only mature muscle fibers rather than satellite cells, myoblasts, and myotubes should be considered a source of myokines; whether proteins, peptides, lipids, steroids, and nucleotides should be included as myokines; and whether myokines should include some molecules leaked from damaged muscle (Fig. 1).

Reported myokines

Table 1 lists the proteins that have been reported as myokines. Only a few reports are available on most myokines, except IL-6.

IL-6

IL-6 is the most extensively examined myokine and is considered to be released from skeletal muscle by muscle contraction. An increase in plasma IL-6 levels after exercise was first reported in the early 1990s[14,15]. However, the source of plasma IL-6 has long been believed to be immune cells aggregated in damaged muscle[16]. This is because plasma IL-6 levels are higher after eccentric exercise, which induces muscle damage, than after concentric exercise[16,17]. However, accumulating data have suggested that muscle contraction without muscle damage also increases plasma IL-6 levels[18-20]. Moreover, plasma IL-6 levels reach a peak at the end of exercise or immediately after it, followed by a rapid decrease to basal levels[18,21,22]. In addition, the increase in plasma IL-6 due to exercise is not always correlated with the degree of muscle damage[19,23,24]. A recent review summarized features of exercise-induced changes in plasma IL-6 using data from 67 exercise trials (800 subjects) in different reports[26]. Exercise was categorized into the following four types in the review: knee-extensor, bicycling, running, and eccentric[25]. The magnitude of the increase in plasma IL-6 is dependent on the intensity and duration of exercise, and plasma IL-6 does not tend to be higher during eccentric exercise than during concentric exercise, suggesting that muscle damage is not the cause of the increase in IL-6

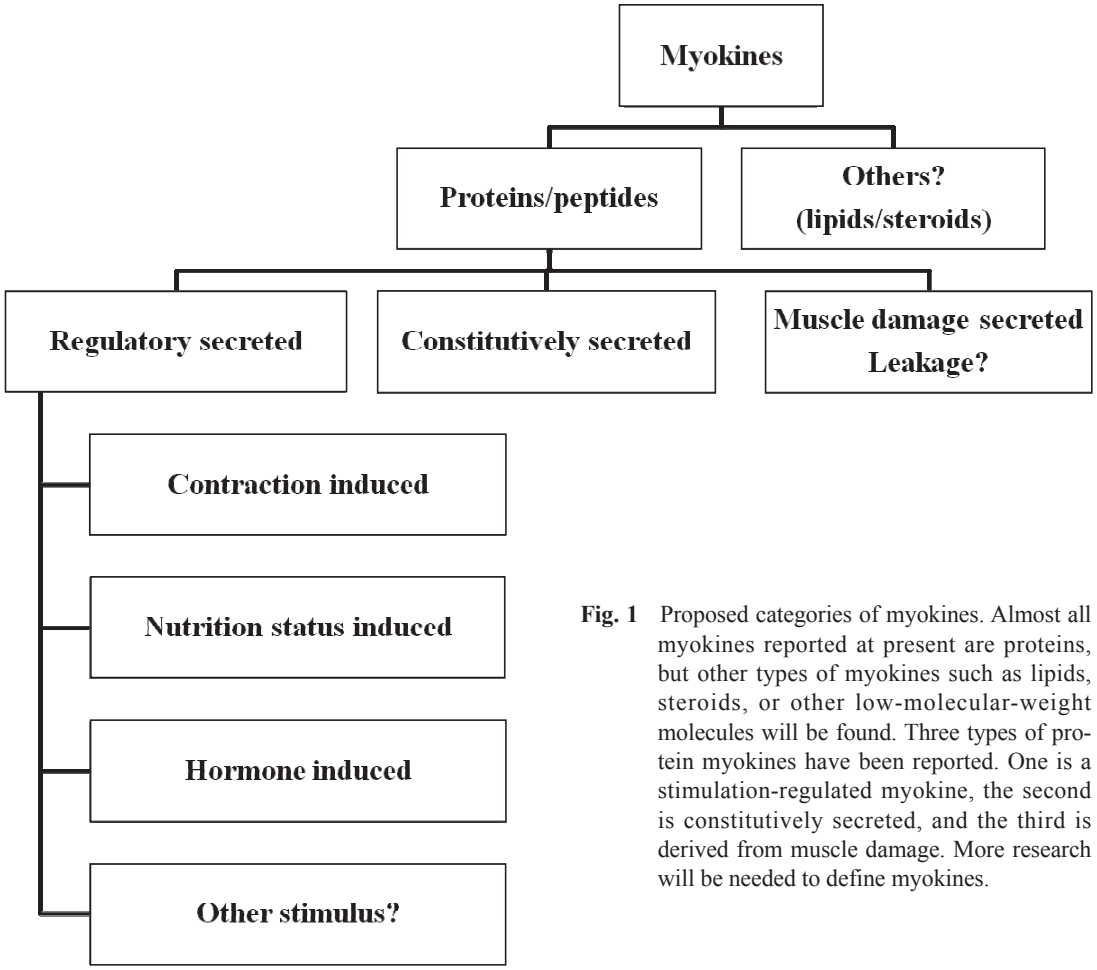

Fig. 1 Proposed categories of myokines. Almost all myokines reported at present are proteins, but other types of myokines such as lipids, steroids, or other low-molecular-weight molecules will be found. Three types of protein myokines have been reported. One is a stimulation-regulated myokine, the second is constitutively secreted, and the third is derived from muscle damage. More research will be needed to define myokines.

after exercise. However, as these studies were conducted with *in vivo* models, it is impossible to neglect the possibility of IL-6 originating from other types of cells surrounding skeletal muscle. Immunostaining approaches such as *in situ* hybridization and immunohistochemistry have shown that prolonged continuous muscle contraction increases IL-6 mRNA and protein in myocytes[26,27]. In addition, IL-6 mRNA is not detectable after exercise in blood mononuclear cells[2], suggesting that muscle cells have the ability to produce and release IL-6. In contrast, one study reported increased IL-6 mRNA in blood mononuclear cells after exercise[28]. Using microdialysis catheters, Langberg et al. found that prolonged physical activity enhances significant IL-6 production in connective tissues around the human Achilles tendon[29]. It was also demonstrated that a session of bicycling exercise for 60 min induced IL-6 release from subcutaneous and abdominal adipose tissue[30]. In addition, exercise increases IL-6 gene expression in adipose tissue[31]. The brain, also, releases IL-6 during cycling exercise[32]; In another study, IL-6 mRNA levels in the hippocampus of mice increased during treadmill running[33]. These reports suggest that many organs could contribute to the source of plasma IL-6 during exercise.

More recently, Nedachi et al. looked at C2C12 myotubes contracted by electrical stimulation and found an increase in IL-6 levels in cell culture medium[34]. This report supports the hypothesis that skeletal muscle is a source of IL-6 released into blood circulation during exercise.

The function of IL-6 released from skeletal muscle during exercise still remains unclear. IL-6 is an inflammatory cytokine and its plasma level increases with infection[35], age[36], and in patients with rheumatoid arthritis[37]. Metabolic diseases such as type II diabetes are also associated with increases in plasma IL-6 concentrations[38-42]. In contrast, some reports suggest that IL-6 plays a positive role in glucose metabolism[8,43,44], increases glucose uptake in myocytes[45], and increases insulin-stimulated glycogen synthesis in skeletal muscle[46]. Some controversial observations have also been reported, such as an IL-6 decrease in insulin sensitivity in skeletal muscle[47], adipocytes[48], and hepatocyte[49]. IL-6 seems to have diverse effects in different tissues. Of note, muscle-specific IL-6 overexpression in mice results in hyperinsulinemia, reduced body weight, and reduced insulin-stimulated glucose uptake[50]. Thus, more studies are needed to elucidate the role of IL-6 released during exercise.

Other myokines

Some studies have reported an increase in the mRNA level due to muscle contraction or exercise as evidence for the presence of myokines. However, an increase in mRNA levels in skeletal muscle does not mean that a coded product is secreted from skeletal muscle. Therefore, this review does not discuss such reports. Similarly, this review does not consider reports that show only an increase in molecules in the plasma as evidence for their secretion, and hence, BDNF[51] and oncostatin M[52], which are relatively popular myokine molecules, are not listed in Table 1.

Chemokine ligand 1 (CXCL1/KC), lipopolysaccharide (LPS)-induced CXC chemokine (CXCL5/LIX), and vascular endothelial growth factor increase in cell culture medium following electrical stimulation-driven contraction of C2C12 myotubes[34,53]. An increase in plasma CXCL1/KC and CXCL5/LIX levels have been confirmed in acute treadmill running mice[34], suggesting that these proteins might have some endocrine functions. IL-8 and IL-15 have been considered possible myokines because these molecules increase during exercise; however, the data were mostly from *in vivo*[54-58] or mRNA studies[59-62]. A recent study by Peterson reported that IL-8 (mouse MIP-2) and granulocyte macrophage colony-stimulating factor (GM-CSF) are secreted by mechanical stretching. In their data, C2C12 myotubes, which were differentiated on a flexible flat-bottom plate and stretched with a vacuum-based system, released IL-8 and GM-CSF under non-injurious and injurious stretching. The authors concluded that these proteins are secreted from skeletal muscle in response to increasing degrees of muscle injury, promoting neutrophil chemotaxis after muscle injury[63]. However, in their data, IL-8 and GM-CSF were detected in cell culture medium containing injured cells; thus, there is no direct evidence that skeletal muscle secretes IL-8 or GM-CSF by non-injurious stretching. Therefore, these molecules may have been leaked from damaged cells during stretching. Similarly, it has not been confirmed whether IL-15 is released from skeletal muscle cells. Alternatively, transgenic mice overexpressing IL-15 in skeletal muscle show increased levels of circulating IL-15 with reduced body fat and increased bone minerals, suggesting that IL-15 released from skeletal muscle has the potential to regulate systemic metabolism[57].

Some myokines are secreted into cell culture medium without any contraction stimulus and affect cells in an autocrine or paracrine manner. For example, IL-7 was detected in human satellite cell culture medium without any stimulation and was supposed to be related to myogenesis[64]. Leukemia inhibitory factor is also secreted into culture medium containing human satellite cells, and this might be related to cell proliferation[65]. Follistatin-like 1 (Fstl1) in C2C12 myotubes is secreted into cell culture medium, and these cells show activation of Akt and endothelial nitric oxide synthase (eNOS) signaling[66]. Intramuscular overexpression of Fstl1 results in increased capillary density and association with eNOS in ischemic hind limbs, suggesting that Fstl1 stimulates revascularization under ischemic stress[66].

Myostatin is a member of the transforming growth factor-beta family and is known to be secreted from skeletal muscle. Myostatin is a negative regulator of skeletal

Table 1. Reported myokines

Myokine	Full name	Species/Source	Stimulus	Ref.
MIF	Macrophage migration inhibitory factor	L6	No stimulation	(*74*)
Musclin	-	C2C12 myocytes	Nutrition status	(*75*)
Fstl1	Follistatin-like 1	C2C12	Ischemic situation (Akt signaling)	(*66*)
IL-6	Interleukin-6	C2C12	Electrically contraction	(*34*)
		Primary human satellite cell	No stimulus	(*64*)
FGF-21	Fibroblast growth factor-21	C2C12	Constitutively active Akt1	(*78*)
CXCL1/KC	Chemokine ligand 1	C2C12	Electrically contraction	(*34*)
CXCL5/LIX	LPS-induced CXC chemokine			
GM-CSF	Granulocyte macrophage colony-stimulating factor	Primary human satellite cell	Mechanically strained	(*63*)
IL-8	Interleukin-8			
PAI-1	Plasminogen activator inhibitor-1	L6 myotubes	insulin	(*10*)
Adiponectin		L6 myotubes	rosiglitazone treatment	(*80*)
Myostatin		Human skeletal muscle cell	No stimulation	(*68*)
IL-15	Interleukin-15	Muscle specific Overexpression mouse	No stimulation	(*57*)
IL-7	Interleukin-7	Primary human satellite cell	No stimulation	(*64*)
LIF	Leukemia inhibitory factor	Human skeletal muscle satellite cell	No stimulation	(*65*)
VEGF	Vascular endothelial growth factor	Rat primary skeletal muscle cell	Electrically contraction	(*53*)
Visfatin		L6 myotubes	No stimulation	(*79*)
ST6GalI	ST6 beta-galactosamide alpha-2,6-sialyltranferase 1	C2C12 Amyloid-beta overexpressed cell	No stimulation	(*88*)
MCP-1/CCL2	Monocyte chemotactic protein-1 /Chemokine ligand 2	C2C12	No stimulation	(*89*)
MCP-2/CCL8	monocyte chemotactic protein-2 /Chemokine ligand 8			
MCP-3/CCL7	Monocyte chemotactic protein-3/Chemokine ligand 7			

*Irisin was newly discovered in January 2012[81] as a myokine. However, the experiments did not directly show that skeletal muscle cells release irisin; therefore, Table I excludes irisin.

muscle mass, and deleting the myostatin gene in mice results in increased skeletal muscle[67]. A preliminary study showed that myostatin only affects muscle development locally or systemically. However, recent data suggest that the mature form of myostatin is secreted into culture medium containing more primary human skeletal muscle cells from obese subjects than from nonobese subjects[68]. Plasma myostatin and myostatin protein expression in skeletal muscle also increase in obese subjects, suggesting that myostatin might be related to energy metabolism or insulin resistance in type II diabetes[68].

Macrophage migration inhibitory factor (MIF), which is an important regulator of inflammation, has recently been shown to be secreted by various types of cells, including macrophages and T cells[69], the anterior pituitary gland[70], heart[71], and testis[72]. Secretion of MIF is reported to be related to glucose metabolism. Pancreatic islets secrete MIF in a glucose-dependent manner[73]. MIF is secreted in response to tumor necrosis factor-alpha (TNF-α) in skeletal muscle cells and induces insulin resistance in peripheral muscle and enhanced glucose uptake and glycolysis in skeletal muscle, suggesting that secreted MIF from skeletal muscle cells is also related to the regulation of glucose metabolism[74]. Musclin is also supposed to be a myokine released from skeletal muscle, and its expression level changes dynamically with nutritional status[75]. FGF-21, which is predominantly expressed in liver and regulates glucose and lipid metabolism[76,77], is secreted from skeletal muscle cells following insulin stimulation[78]. Secretion of FGF-21 increases in Akt1-overexpressing C2C12 myotubes. Serum FGF-21 levels also increase in Akt1-overexpressing mice, suggesting that activation of the PI-3-Akt signaling pathway is related to FGF-21 secretion. From proteomic analysis, Yoon et al. found 14 proteins that increased in L6 cell culture medium following insulin stimulation[10]. To verify the proteomic results, they performed Western blotting and found that matrix metalloproteinase-2 and plasminogen activator inhibitor-1 were actually released from skeletal muscle in response to insulin stimulation[10]. These data suggest that insulin also stimulates secretion of some myokines.

Visfatin, which is an adipokine, is found in L6 cell culture medium[79]. Although circulating visfatin levels are positively correlated with skeletal muscle weights in rats, the biological function of secreted visfatin from skeletal muscles cells remains unclear. Adiponectin, which is also an adipokine, is induced by treatment with the antidiabetic drug rosiglitazone in rat L6 muscle cells[80]. Although the functions of these myokines are unclear, it is intriguing that there must be more complex communication among organs. More recently, a myokine named "irisin" was discovered[81]. Irisin affects white adipose cells and stimulates uncoupling protein 1 expression and induces browning of white fat, resulting in increased energy expenditure and improvement in high fat-induced insulin resistance. In addition, it increases in plasma following

endurance exercise, suggesting that irisin might be a myokine that explains the health benefits of exercise.

Myokine secretion mechanism

Skeletal muscle has only recently been considered as a secretory organ. For this reason, its secretion mechanism is poorly understood.

Adipose tissue has long been considered a tissue for storing lipid as an energy source. It has become clear that adipocytes secrete several hormones (adipokines). Until now, more than 100 adipokines, including TNF-α, IL-6, resistin, leptin, and adiponectin, have been reported[82]. Leptin is a well-known adipokine whose level is correlated with body fat mass and body mass index[83]. Recent data show that leptin is secreted by both regulatory (insulin stimulated) and constitutive pathways[82,84]. Ye et al. reported that leptin and resistin are secreted by both regulatory and constitutive pathways[85]. Insulin-mediated secretion of leptin is regulated by exocytotic release mechanisms, and this is inhibited by forskolin, a protein kinase A activator[86]. Secretion of resistin is inhibited by insulin stimulation and facilitated by forskolin[85]. Regulated vesicular secretion is usually calcium dependent. However, their report showed that constitutive secretion of these adipokines is calcium dependent, whereas regulated secretion is calcium independent[85]. Adiponectin and leptin are localized in different compartments, and their secretion pathways are also different, although both proteins are secreted by insulin stimulation[87]. Similar secretion mechanisms might be considered for myokines, although details are yet to be revealed in a constitutive pathway or insulin- and contraction-regulated pathways. It will certainly be necessary to study the intracellular storage, vesicle trafficking, and regulatory signals of myokine secretion, which will lead to an understanding of not only how myokines are secreted but also how they respond to various types of metabolic signals.

Conclusion

It has long been considered that skeletal muscle releases some hormones during exercise because the levels of many plasma cytokines change drastically by exercise. However, because a conventional cell culture system cannot be utilized for myokine study due to a lack of muscle contractile activity, it will take time to confirm that skeletal muscle releases hormones. The recent development of a cell contraction system has high potential to solve this problem; this system has proven that skeletal muscle does secrete some proteins[34]. Although only a few proteins have been confirmed to be directly released from skeletal muscle cells, these observations allow us to consider skeletal muscle as a secretory organ. The definition of myokines is still obscure. In addition, the secretory mechanism and physiological functions of myokines are still unclear. As myokine research is still in its initial stage, it will be important to carefully interpret whether newly reported myokines are released from skeletal muscle itself.

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