# Myokines: Discovery Challenges and Therapeutic Impediments

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## Abstract

Physical activity has been linked with maintenance of bodily health and prevention of numerous pathologies which might arise in its absence. Physical surge renders the skeletal muscles of the body to come into action which then, besides performing the basic functions of locomotion and maintenance of body posture, produce various proteinaceous secretions. These secretions contain skeletal muscle-released biochemical products known as myokines. More than 650 myokines have been identified and this list is increasing so rapidly that this branch of proteomics can now be designated as a 'myokinome'. This review was planned to describe the major challenges faced in myokine discovery, barriers in use of myokines as a treatment modality and the techniques utilised to overcome these scientific hurdles. Establishment of correlation of myokines with the state of health and disease can help improve clinical diagnostics and will ultimately improve the quality of life of patients.


**Keywords:** Exercise, Physical inactivity, Muscle, Skeletal muscle, Myokines.

## Introduction

Muscle is a highly specialised tissue of mesodermal origin and has the fundamental property of contractility. The primary function attributed to muscle is the allowance of various body movements. Muscles give general form to the body and their contraction is associated with generation of heat which regulates body temperature. Their activity also assists blood circulation and internal organs' functioning.[1] Broadly, muscles can be classified into 3 categories in which each type shows morphological and functional similarities as well as differences with the other two. These are the skeletal, cardiac and smooth types of muscles. Skeletal type accounts for the major bulk of muscles in the body. Skeletal muscles are innervated by somatic motor nerves and are composed of parallel bundles of long multinucleated fibres, which, in turn, are made up of myofilaments, namely actin, myosin and tropomyosin. Skeletal muscles exhibit cross-striations under the microscope. This muscle variety is further subdivided into 2 types based on their contractility. The first one is the slow twitch or red fibres or type I fibres, and the second one is the fast twitch or white fibres or type II fibres.[1,2]

Physically inactive people have a life span that is relatively shorter than those of physically active people.[3] It has been reported that physical inactivity is the cause of increased prevalence of various chronic diseases among the human population. It increases the risk of obesity,[4] cardiovascular diseases[5] and cancers, including colon cancer,[6] as well as postmenopausal breast cancer.[7] This can be regarded as "the diseasome of physical inactivity".[8] The underlying pathophysiology is related directly to physical inactivity that leads to the accumulation of visceral fat in the body which consequently causes the activation of macrophage infiltration into those particular fat deposits. This activation causes the initiation of chronic systemic inflammation which leads to the occurrence of the above-mentioned diseases.[4] A comparison of the widespread effects of physical activity and physical inactivity is interesting (Table-1).

**Table-1:** Effects of Physical Activity versus Physical Inactivity on the human body.

| Physical Activity | |
|---|---|
| Improved insulin sensitivity | |
| Improved metabolic processes | Maintenance of muscle mass |
| Reduced blood pressure | Increased cerebral blood flow |
| Reduced inflammation | Improved brain oxygenation |
| Reduced adiposity | Better heat dissipation via increased sweet |
| Increased cardiorespiratory fitness | rate |
| Reduced circulating lipids | Decreased liver fat accumulation |

| Physical Inactivity | |
|---|---|
| Decreased insulin sensitivity | Colon cancer |
| Reduced postprandial lipid clearance | Post-menopausal breast cancer |
| Loss of muscle mass | Pancreatic, liver & endometrial cancer |
| Accumulation of visceral adiposity (Obesity) | Renal cell cancer |
| Type-2 diabetes mellitus | Esophageal adenocarcinoma |
| Metabolic syndrome | Osteoporosis |
| Mental diseases (Dementia & Depression) | Osteoarthritis |
| Cardiovascular diseases (CVDs) | Erectile dysfunction |
| | Polycystic ovarian syndrome |

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Unlike physical inactivity, regular exercise is regarded as an essential component that helps in promoting the overall health of the body. The entire human body is affected by exercise, but the most important organ activated as a result of this activity is the skeletal muscle.[9] Exercise causes reduction in blood pressure,[10] inflammatory status[11] as well as improvement in blood lipid profile of an individual.[12] In this regard, a class of proteins secreted by the skeletal muscle fibres which exerts both local and systemic effects is known as myokines. Myokines have also been called 'exercise factors' as they are released in the circulation as a consequence of physical activity. Recent studies, however, classify exercise factors as a subset within the myokine family because not all myokines are produced as a result of physical surge and not all of them have systemic function. Research also indicates that myokines can also be produced from other body tissues, like adipose tissue, and thus cannot be labelled as a secretory product of skeletal muscle alone.[9]

Myokines vary in their molecular weight (MW) usually lying in between the range of approximately 5-20 kilo Daltons (kDa).[13] Myokines have got an impact on multiple muscular functions, especially controlling muscle hypertrophy, regeneration and metabolism.[13,14] The activation of calcium signalling pathway or change within the energy status of a particular muscle fibrer usually leads to the release of myokines during or after exercise.[15] The first myokine to be discovered was myostatin[16] whereas interleukin 6 (IL6) was the first one found to be secreted into the blood as a consequence of muscle contraction.[15] Until now, approximately 635 secreted proteins of skeletal muscle origin have been identified. This large list of myokines includes approximately 35 growth factors, 40 cytokines and 36 metallo-peptidases.[17] These proteins can act as strong signalling mediators to other cells and tissues of the body. Skeletal muscle is a dynamic organ which is involved primarily in locomotion, generation of body heat and also in maintenance of metabolic homeostasis.[8,17] Myokines exert effects on various organs of the body, such as liver, pancreas, bone, heart, fatty tissue and brain cells. These functions enhance the role of skeletal muscle as a vital secretory organ.[13] Muscle secretome has also been analysed by induction of contraction in the skeletal muscle myotubes through electric pulse stimulation (EPS). This particular technique has contributed to the discovery of 52 novel myokines among which 48 were shown to be released by contractile activity alone.[18,19] Myokine production is governed by a number of growth factors such as transforming growth factor beta (TGF-β) and insulin-like growth factors (IGFs) which control its proliferation and differentiation. TGF-β inhibits whereas IGFs promote differentiation.[20-22]

**Table-2:** General functions of myokines.[25]

| General Functions of Myokines | |
|---|---|
| Control of mechanism of tissue repair | Satellite cell proliferation and migration |
| Control on Inflammatory cascade | Extracellular matrix organization |
| Influence on mechanism of cell adhesion | Extracellular matrix disassembly |
| Anatomical structural development | Positive regulation of locomotion |
| Regulation of angiogenesis | Modulation of platelet degranulation |
| Effect on host immune response | Regulation of fatty acid oxidation |
| Control of insulin secretion | Anti-tumour defense mechanism |

## General Functions Attributed to Myokines

A range of potent effects on various tissues of the body are mediated by the myokines. A few of these effects are on the skeletal muscle itself designated as the 'autocrine' function, a few to the tissues present at a small distance known as the 'paracrine function', and others controlled through an 'endocrine function' in which these molecules enter the blood stream and act on tissues present at a long distance from their site of production (Table-2).[15]

## Challenges in Myokine Discovery and Technological Advents

A number of factors have been identified that serve as obstacles in myokine discovery and their validation. These include the consideration of small sample sizes in various studies, the use of low statistical power, unavailability of accurate measurement assays, and also the biology of exercise itself is affected by a range of factors such as the duration, nature, frequency and the surrounding environmental conditions.[15,23] It has also been found that a number of myokines are released in every minute concentrations of picomole or femtomole in the immediate vicinity of the muscle and therefore they might never reach the circulating body fluids. So, for this purpose, if plasma or serum is monitored for these specific myokines, the results will turn out to be unsatisfactory.[24,25] All of these barriers together need to be surpassed so that a better understanding of myokine functioning can be made, starting from their site of origin and then extending to their diverse actions elsewhere

in the body.

With the advent of modern technology, the techniques and methodologies used for myokine identification and isolation have improved manifold. A few of them incorporated readily are cell culture models,[17] microarray-based analysis of secreted proteins,[26] mass spectrometry-based proteomic analysis,[27] stable isotope labelling by amino acids in cell culture (SILAC),[28] gene ontology (GO), immune-staining, real-time Quantitative polymerase chain reaction (Q-PCR), whole cell extracts and Western Blotting and Zymography.[17] More recently, liquid chromatography tandem mass spectrometry (LC-MS/MS) has also been used for this purpose.[23]

Although studies have been published on molecules such as IL6 (Interleukin-6) as an inflammatory marker and IGF-1 (Insulin like growth factor-1) as an anxiolytic and antidepressant marker in diseases such as coronary artery disease (CAD) and obsessive compulsive disorder (OCD) in Pakistan and India respectively,[29,30] to our knowledge, no work has been published related to such and other molecules in the subcontinent with their particular roles as myokines.

**Impediment in the use of Myokines as a Therapeutic Product**

There are many reasons attributed to myokines which makes it difficult for them to be administered to the patients as a treatment option. The prime issue being that all myokines are proteins in nature and have got physiochemical as well as proteolytic instability. The other barriers are their limited solubility content, short half-life in plasma, immunogenicity and toxicity.[31,32] For this purpose, the protein biopharmaceuticals have introduced techniques based on polymerised ethelene glycol (PEG) called PEGylation which is a process of covalently adding repeating units of PEG to proteins[33] and crystallisable fragment (Fc) fusion which is a process of adding Fc domain of immunoglobulin G (IgG) molecules to proteins[34] to improve the therapeutic performance and utility of these myokines, particularly in terms of their stability, pharmacokinetics and pharmaco-dynamics.

Moreover, another area of concern is to achieve cell or tissue-specific targeting of myokines to prevent any possible side effects. Myokines, as discussed, are also secreted by tissues other than the skeletal muscle. This is the reason they have got their receptors elsewhere in the body too. Incorporation of drug delivery by nanotechnology might help achieve this particular constraint.[35]

**Future Endeavours in the field of Myokinomics**

The discovery of myokines paves the way for new scientific, technological and scholarly purviews. Individualised studies done on the muscle secretome have added to the identification and isolation of novel myokines and this list keeps on increasing. Skeletal muscle can now be designated as an 'endocrine organ'. The production of numerous myokines from skeletal muscles suggests how these molecules are responsible for taking part in various bodily processes and activities. Research on various functions of myokines may further help us understand the role of muscle as an immunogenic organ. With the rapid pace of the ongoing research in this field, it is surely believed that myokine as a separate specialty will be the prime choice of medical researchers in the near future.

Myokines produced by the skeletal muscle fibrers have widespread body functions affecting multiple body organs, the important ones being the brain, liver and adipose tissue. The establishment of an appropriate crosstalk between various body cells and tissues requires the release of secreted factors which then elicit specific cellular responses in the target cells. Myokines specifically act in a similar manner and perform the functions as explained above. The diversity of multiple identified proteins which are released by the skeletal muscle indicates the range of effects they exert and the numerous processes they control. The most important one is the control of the growth factors' functioning, cytokine and also the enzymatic activity in the body.

The role of myokines in the pathogenesis of 'oral and maxillofacial neoplasms' is less understood, thus this oncological specialty provides an essential area for future research. Myokines have been studied in adjunction with the stem cell therapy where they might act as growth initiators or retarders. The myogenic influence on the control of the diverse body functions demands further investigations where the whole myokine-body interplay may be termed 'the myogenic programme'. This could help scientists understand and develop novel therapeutic regimens for the cure of multiple pathological disorders that are a result of myokine malfunctioning. Besides, these secreted proteins could serve as molecules modulating the activity of other

organs of the body.

Ongoing research work on one of the novel myokines has shown alterations in its level in saliva of patients of chronic periodontitis compared to the healthy individuals, paving the way for establishing this myokine as one of the biological markers for chronic periodontitis.

## Conclusion

Myokines are biological factors which are of prime importance in modern scientific era due to their widespread and vital effects in the functioning of the human body. In this regard, their appropriate identification and utilisation as a treatment modality will contribute greatly to the field of medicine.

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