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Myokines as a Promising Marker of Metabolic Disorders and Physical Activity

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Abstract. Currently, about 82 myokines identified and their number is increasing. It is shown that the major regulator of myokine expression and production is exercise. The expression level of IL-6 is dependent on the amount of muscle mass involved in contraction. It is assumed that the decrease in the partial pressure of oxygen, the increase in $[Ca^{2+}]_i$ ratio and AMP/ATP (exercise response) are major regulator of transcriptome and proteome changes in the skeletal muscle cells, including a myokine set.

Skeletal muscles represent up to 40% of the total body mass and contain 50-75% of all body proteins. As a part of the musculoskeletal system it maintains posture, body movement in space etc. During the last 2 decades it was shown that skeletal muscles function as an exercise-dependent endocrine organ secreting dozens of cytokines, i.e. glycoproteins with molecular weights of 15-30 kDa [20]. By analogy with adipokines and hepatokines, i.e. proteins secreted by adipocytes and hepatocytes, respectively, proteins produced by skeletal muscles were classified as myokines. These muscle-derived secretory proteins exert auto-, para- or endocrine effects communicating with other organs, such as adipose tissue, liver, bone, immune system [21]. In 1990th-2000th several investigations demonstrated that during exercise plasma interleukin-6 (IL-6) was transiently increased up to 100-fold. Importantly, unlike sepsis-induced production of this cytokine the sharp increment of IL-6 evoked by exercise was not preceded by elevation of circulating tumor necrosis factor (TNF)-α. Pedersen and co-workers were the first who found that exercise does not affect IL-6 mRNA content in monocytes thus ruling out the possible implication of immune system cells. Keller and co-workers reported that both IL6 mRNA and immunoreactive protein content are increased in human contracting skeletal muscle. They also found augmented IL6 transcription rate in nuclei isolated from human muscle biopsies after the onset of exercise. Viewed collectively, these experiments demonstrated that myoblasts rather other type of cells presented in skeletal muscle and neighboring tissues are the major source of IL-6 [21].

Recent proteomics studies indentified more than 500 proteins secreted by human and rodent skeletal muscle cells. Studies in recent years have allowed representing the classical ideas about the functions of organs and tissues. Since the beginning of the two thousandth's in *in vivo* studies have shown that muscle cells are capable of releasing proteins and peptides which affect the functional activity of the cells of other tissues – "myokines" [19].

Secretome studies include the search and verification of the novel proteins and peptides, synthesized by muscle cells [4]. Currently, about 82 myokines identified [22] and their number continues to increase. The mechanisms of myokine expression, production and secretion discussed in many papers. It is assumed that the decrease in the partial pressure of oxygen, the increase in $[Ca^{2+}]_i$ ratio and AMP/ATP (exercise response) are major regulator of transcriptome and proteome changes in the skeletal muscle cells, including a myokine set. It is known that the exercise with the above factors is accompanied by a 3-4-fold Na^+_i concentration increase (because of permanent

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activation of voltage-dependent ion channels) [11]. Ion homeostasis changes of skeletal muscle during exercise are so significant that lead to 2 fold increase in K + concentration in the venous blood.

An analysis of the original articles showed that the majority of researchers employed in the experiment long dynamic load – running exercise, bicycle, swimming. It is shown that long cyclic loads stimulate the expression of IL-6 [23], IL-8 [5], IL-15 [26], PGE2 [10]. The myostatin concentration in mice muscle cells is reduced after the running load, thereby stimulating the growth and differentiation of satellite cells [9]. The static loads rarely use in experimental studies. For example, no change in the concentration of PGE2 has allowed suggesting that prolonged exercise in the mode of moderate power contribute to more significant changes in intracellular environment of muscle tissue [10]. Submaximal power exercise does not lead to a change in the concentration of eNOS, and endurance training contributes to its increase [7]. Later it was shown that interval of training increase the eNOS concentration [6]. The increase in some myokine concentration occurs in the recovery period. For example, increased COX-1 and COX-2 occurs after performing strength exercises [3]. A single cyclic exercise is sufficient for CXCL1 production [18], but repetitive exercise is important for accumulation LIF concentration [1]. In experimental studies on muscle cell cultures using an electric pulse stimulation (EPS) and mechanical strength for the simulation exercise. In experiments using different power and EPS pacing rate registered an increase LIF and CXCL1 (human skeletal muscle cell culture / hSkMC) [1; 24], CXCL1 and IL6 (mice muscle cell culture / C2C12) [15; 16], COX 2 (rat muscle cell culture / L6) [14]. Endocrine, paracrine and autocrine effects of myokines studies by many authors. Most of the papers are considered the impact of myokines on adipose and bone tissue, liver, pancreas and vascular endothelium [18]. It proved that LIF, IL-4, IL-6, IL-7 and IL-15 promote muscle hypertrophy and myogenesis. Myostatin inhibits muscle hypertrophy, but physical activity releases of myostatin inhibitor by liver – follistatin. It is found that BDNF and IL-6 promotes the AMPK-mediated oxidation of fats and IL-8 can participate in angiogenesis mediated by physical load. In general, IL-6 has systemic effects and influences on the liver, adipose tissue and increased insulin secretion through the regulation of GLP-1.

Some molecules are both myokines and adipokines associated with obesity and insulin resistance – IL-6, IL-7, IL-8, MCP-1, PEDF. The concentration of IL-6 in the plasma depends on the content of the fatty component and it is elevated in type 2diabetes, as well as increased expression of IL-8 in visceral adipose tissue. In turn, a sedentary lifestyle provokes violations of myokine production, as well as resistance to their effects, which leads to an increased risk of metabolic disorders and diseases of the cardiovascular system. It is known that contraction of skeletal muscle is induced by propagation of action potential: opening of voltage-sensitive Na^+ channels (Na_v), sarcolemma depolarization from the resting potential (E_m) of -80 mV to +30 mV, conformation transition of the skeletal muscle isoform of voltage-sensitive L-type Ca^{2+} channels (Ca_v), also known as dihydropyridine receptors (DHPR), and its physical interaction with the skeletal muscle isoform of the ryanodine receptor Ca^{2+} release channels (RyR). Activation of RyR leads to Ca^{2+} release from the sarcoplasmic reticulum, elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), $Ca^{2+}{}_i$ binding to troponin that, in turn, results in activation of myosin ATPase and shortening of sarcomeres [13].

Our model suggests that elevation of the $[Na^+]_i/[K^+]_i$ ratio affect myokine transcription and translation independently of elevation of $[Ca^{2+}]_i$ via interaction of $Na^+{}_i$ and $K^+{}_i$ with their hypothetical sensors NaS and KS, respectively. It should be noted that the molecular origin of monovalent cation sensors distinct from ion transporters is still a mystery. This uncertainty is in contrast with rapid progress in the identification of $Ca^{2+}{}_i$ sensors. It should be noted, however, that high-affinity binding sites, initially detected in parvalbumins and calmodulin, are formed by a highly conservative linear amino acid sequence consisting of 14 amino acid residues (the so-called "EF-hand" domain). This knowledge led to the rapid identification of more than 30 other $Ca^{2+}{}_i$ sensors by the screening of cDNA libraries [7]. In contrast, monovalent ion sensors are probably formed by 3D protein structures and recruit space-separated amino acid residues. In addition, high-affinity $Ca^{2+}{}_i$ sensors are almost completely saturated at $[Ca^{2+}]_i$ of 1 μM. This feature led to the identification of amino acid residues by ^{45}Ca binding assay. In contrast to Ca^{2+}, monovalent cations affect cellular function in the millimolar range that complicates their identification by screening with radioisotopes. Recently, Ono and co-workers reported that at the baseline level of $[Ca^{2+}]_i$ (~100 nM), Na^+ interacts with calpain Ca^{2+}-binding sites, and this enzyme functions as $Na^+{}_i$-dependent protease with $K_{0.5}$ of 15 mM for Na^+. Additional experiments should be performed to examine the role of Ca^{2+}-binding proteins as $[Na^+]_i$ sensors involved in transcriptomic and proteomic changes triggered by elevation of the $[Na^+]_i/[K^+]_i$ ratio [18].

It is generally accepted that transcription is under the control of transcription factors interacting with specific response elements within 5'- and 3'-untranslated region (UTR). Considering this, we tried to find Na^+ response element (NaRE) within c-Fos promoter. With the construct containing CRE and all other known transcription elements of the c-Fos promoter, we failed to detect any significant elevation of luciferase expression in HeLa cells

subjected to 6-hr inhibition of Na^+/K^+-ATPase that contrasted with massive accumulation of endogenous c-Fos mRNA and immunoreactive protein in ouabain-treated HeLa cells.

Several hypotheses could be proposed to explain negative results obtained in this study. (*i*) NaRE is located within introns or/and the c-Fos 3'-UTR. (*ii*) $[Na^+]_i/[K^+]_i$ ratio elevation affects gene expression via epigenetic modification of the DNA, histones or nucleosome remodeling, i.e. regulatory mechanism having a major impact on diverse cellular functions. Importantly, the epigenetic mechanism of gene expression does not contribute to the regulation of L-luc transcription in the plasmid employed in our experiments. (*iii*) Increasing evidence indicates that gene activation or silencing is under the complex control of three-dimensional (3D) positioning of genetic materials and chromatin in the nuclear space. It may be proposed that augmented $[Na^+]_i/[K^+]_i$ ratio affects gene transcription by changing the 3D organization of DNA-chromatin complex. These hypotheses will be verified in forthcoming studies. It should be underlined that side-by-side with transcription, translation and secretion stages triggered the gain of Na^+_i, Ca^{2+}_i and loss of K^+_i myokine can affects their production by autocrine receptor-mediated mechanisms. Thus, IL-15 augments expression of peroxisome proliferator-activated receptor δ (PPARδ) and silent regulator of transcription-1 (SIRT1) via its interaction with IL-15 receptor IL-15Rα, PGE_2 triggers accumulation of IL-6 whereas CXL-1 expression is regulated by IL-6 [5]. Comparative analysis of exercise-sensitive secretome of skeletal muscle cells and selected myokines revealed little correlation between mRNA and protein levels, indicating pronounced modulation of myokine translation and/or secretion by unknown intermediates of intracellular signaling. Data considered below strongly suggest that besides of transcription elevation of the $[Na^+]_i/[K^+]_i$ ratio seen in contracting skeletal muscles affects translation of myokines. Almost 50 years ago, it was demonstrated that protein synthesis in prokaryotes is sharply inhibited in the absence of K^+. Later on, the requirement of K^+ for protein synthesis was detected in animal cells of different origins [17]. In human fibroblasts, it has been shown that sustained Na^+/K^+-ATPase inhibition suppresses protein synthesis without any impact on transcription, ATP content and amino acid transport, indicating a direct influence of $[K^+]_i$ on the protein synthesis machinery. In reticulocytes, globin contributes to more than 90% of total protein synthesis. In these cells, it was found that K^+_i depletion inhibits the elongation step of globin synthesis without any impact on ribosome subunit assembly. Half-maximal activation of globin synthesis by reticulocyte lysate in medium containing 60, 90 and 125 mM Na^+ was observed at $[K^+]$ of 15, 25 and 40 mM, respectively. These data indicate that elevation of $[Na^+]_i$ diminishes the efficiency of protein synthesis regulation by K^+_i via attenuation of K^+ interaction with its hypothetical sensor. As alternative hypothesis it might be proposed that elevation of $[Na^+]_i$ diminishes the transcription of elongation factors.

It is generally accepted that myokine secretion is mediated by exocytosis [17]. Exocytosis consists of multiple kinetically defined stages such as recruitment, targeting, tethering and docking of secretory vesicles with the sarcolemma, priming the fusion machinery and finally membrane fusion. The final stage is triggered by Ca^{2+} and involves several secretory vesicle proteins including Ca^{2+}-sensing protein synaptotagmin 1 (SYT1). These data suggest that elevation of $[Ca^{2+}]_i$ in contracting muscle may affect myokine secretion independently on regulation of their transcription and translation. Importantly, in addition to Ca^{2+} as a universal regulator, exocytosis my be regulated by intermediates of intracellular signaling triggered by cyclic nucleotides, diacylglycerol and inositol 1,4,5-triphosphate such as cAMP-binding protein EPAC, guanine-exchange factors (Rap1-CEFs). In vascular smooth muscle and endothelial cells, sustained inhibition of the Na^+,K^+-ATPase affected expression of dozen proteins involved in these signaling cascades (data prepared for publication). The role of elevated $[Na^+]_i/[K^+]_i$ ratio in regulation of myokine secretion via altered expression of $[Na^+]_i/[K^+]_i$-sensitive genes involved in the skeletal muscle secretory machinery remains unknown [22]. Data summarized in our mini-review show that side-by-side with canonical Ca^{2+}_i- AMPK- and HIF-1α-mediated signaling pathways, myokine production by contracting skeletal muscle may be mediated by the novel $[Na^+]_i/[K^+]_i$-sensitive, Ca^{2+}_i-independent mechanism of excitation-transcription coupling [22].

Thus, in current research of myokine expression and production are mainly used cyclic typical exercise. Currently there are only fragmented data in experiments on the use of cyclic loads of different power and intensity (training mode moderate power), static exercise, the use of continuous and intermittent exercise. Not found data about the study myokine production during dynamic exercise in maximal, submaximal and high power zones, in some modes of sports training. Also, no data about the differences in the myokine expression studies at different modes of physical activity. However, it is known that morphological and chemical changes in muscle fibers occur depending on the muscle stimulation rate [8, 12, 13, 23]. Furthermore, it has been suggested that proteins secretion affects laminar voltage, tangential force exerted on the surface of endothelial streams [25] (exercise provides change system and regional blood flow). Therefore, it is important to study the myokine production depending on the nature of the exercise, its duration, the presence or lack of rest intervals, fitness level (degree of adaptation to stress the different nature).

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