



Molecular Mechanisms Mediating Adaptation to Exercise

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Abstract

Several experimental and human studies documented the preventive and therapeutic effects of exercise on the normal physiological function of different body systems during aging as well as various diseases. Recent studies using cellular and molecular (biochemical, proteomics, and genomics) techniques indicated that exercise modifies intracellular and extracellular signaling and pathways. In addition, *in vivo* or *in vitro* experiments, particularly, using knockout and transgenic animals, helped to mimic physiological conditions during and after exercise. According to the findings of these studies, some important signaling pathways modulated by exercise are Ca^{2+} -dependent calcineurin/activated nuclear factor of activated T-cells, mammalian target of rapamycin, myostatin/Smad,

and AMP-activated protein kinase regulation of peroxisome proliferator-activated receptor-gamma coactivator 1-alpha. Such modulations contribute to cell adaptation and remodeling of muscle fiber type in response to exercise. Despite great improvement in this field, there are still several unanswered questions as well as unfixed issues concerning clinical trials' biases and limitations. Nevertheless, designing multi-center standard clinical trials while considering individual variability and the exercise modality and duration will improve the perspective we have on the mechanisms mediating adaptation to exercise and final outcomes.

Keywords

Exercise · Physical activity · Molecular mechanism · Signal transduction · Molecular adaptation

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3.1 Background

Several animal and human experiments (both *in vivo* and *in vitro*) as well as clinical evidence documented the beneficial effects of exercise and physical activity on disease prevention or rehabilitation. In addition, exercise and physical trainings are necessary to maintain normal physiological function of musculoskeletal, cardiovascular, nervous, endocrine, and respiratory

systems in geriatrics, and influence the aging process [1]. During recent decades, various investigations described the biological adaptation and revealed modification of intra- and inter-organ communications after physical activity and training. Human studies indicated that these adaptational changes in biological system may differ based on physical/training activity type, severity, duration of each session, and acute or chronic nature as well as sex, age, disease state, genetics of the one who undertakes the practice, and environmental epigenetic factors (i.e., lifestyle, nutritional condition, and physical fitness) [2].

Exercise induces several physiological adaptive processes by modulating cellular and molecular regulatory mechanisms. Modification of molecular pathways including intracellular and extracellular signaling may be attributed to alteration of gene/protein expressions leading to cellular/tissue phenotypic changes. In addition, the role of immune and satellite cells in muscle regeneration and restoration as well as hereditary genetic differences are the most important factors affecting exercise-induced physiological adaptive processes.

Cellular and molecular investigation of exercise physiology started in 1962 by the examination of human skeletal muscle biopsies. Advances in proteomics, genomics, and bioinformatics investigations helped to reveal some cellular and molecular aspects of adaptations to exercise. Evaluation of the skeletal muscle gene expression using computational approaches resulted in identifying about 300 secretory proteins involved in cell signal transduction pathways [3]. Another study showed that 6-week endurance exercise affected the expression of about 800 human muscle genes, out of which 100 genes were related to greater aerobic exercise-induced molecular adaptation. Also, in training-responsive transcriptome, there were three overrepresented DNA sequence representing *PAX3*, *RUNX1*, and *SOX9* transcription factor binding sites, while miRNA targeting these genes were downregulated after aerobic exercise [4]. Moreover, different experimental diseases and knockout (KO) and transgenic animal models were employed to determine exercise-mediated signal transduction modifications.

Exercise-induced molecular adaptation resulted in supra-molecular changes in biological systems which are summarized in Fig. 3.1.

3.2 Skeletal Muscle Adaptations

Since locomotor system has a key role in movement and escape reaction, its status and function are important for human body homeostasis and survival. Although several organs take part in physical activity, muscles are key elements in motor performance and related adaptive responses. Muscle is the main target of exercise-induced mechanical, oxidative, and metabolic stress. Moreover, physical training induces homeostasis disturbance by enhancement of muscle energy storage consumption and increment of cell energy turnover [3]. In muscles, mitochondrial (and other organelles') production of nitrogen and oxygen species, as main intracellular messengers, and norepinephrine, epinephrine, growth hormone, cytokines, cortisol, and calcium, as main extracellular messengers, was shown to activate signaling pathways and compensatory mechanisms mediating adaptation [5, 6]. These messengers alter the biological action of cytoplasmic proteins and enzymes, membrane ion channels and receptors which consequently results in activation or inactivation of signal transduction pathways by phosphorylation or dephosphorylation of the related enzymes and proteins. Cellular and molecular investigations started from 1985 explained some effectors involved in these signaling network and adaptational response. The main proposed signaling pathways in muscles are Ca^{2+} -dependent calcineurin/activated nuclear factor of activated T-cells (NFAT) pathway, mammalian target of rapamycin (mTOR), myostatin/Smad, and AMP-activated protein kinase (AMPK) regulation of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1- α). The cross talk among these pathways was shown to modulate muscle adaptation and cause muscle fiber type remodeling in response to exercise.

During the last decade, transcriptomics, as an approach to examine RNA molecules changes in one or a group of cells, was employed to study

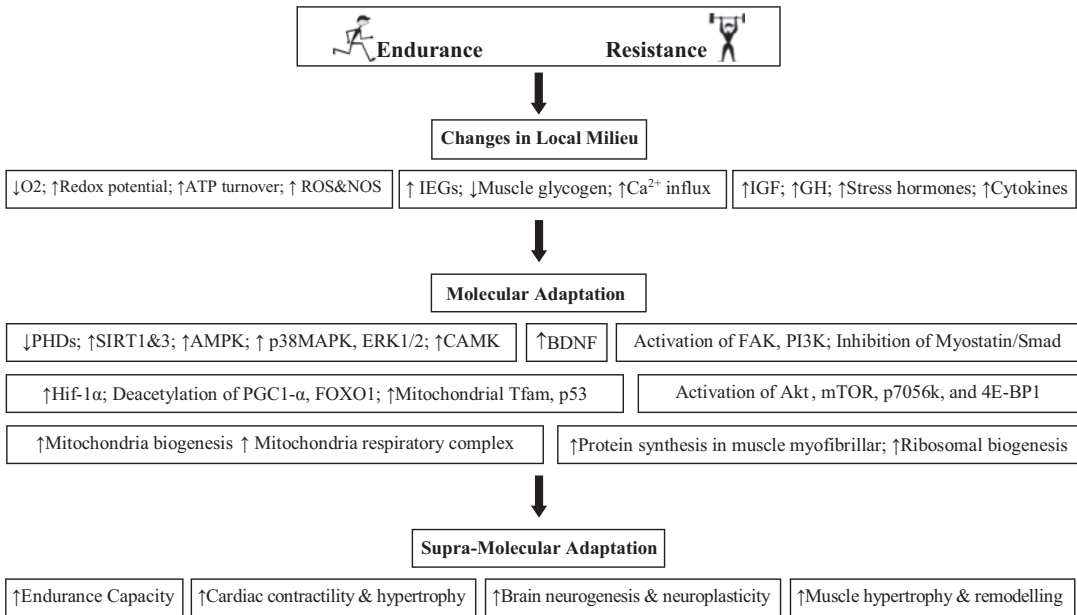


Fig. 3.1 Exercise-induced molecular adaptation resulted in supra-molecular changes in biological systems

the physiological adaptation of human muscle to acute or chronic exercise [7].

3.2.1 Exercise-Induced Signal Transduction Pathways

3.2.1.1 Ca²⁺-Dependent Calcineurin/Activated NFAT Pathway

Elevation of intracellular Ca²⁺ concentration was demonstrated to result in muscle contraction and activation of calmodulin calcineurin/NFAT signal transduction which mediates transformation of muscle fiber type fast to slow. In adult skeletal muscle, this signaling pathway is necessary for maintenance of slow fibers. Phosphatase calcineurin regulates five transcription factors (NFATc1–4 and NFAT5) belonging to NFAT family proteins [8]. In adult muscle, the main NFAT isoform is NFATc1 [9]. Using NFATc1-null mice, the effect of exercise on muscle fiber remodeling was examined and the role of NFATc1 as a transcription factor that induces transformation of fast fiber to slow ones was indicated. In addition, it was demonstrated that NFATc1 might interact with the activation domain of MyoD which leads to blockage of the essential

transcriptional coactivator p300 recruitment and inhibition of promoters of the MyoD-dependent fast fiber gene [10].

Ca²⁺ release from ryanodine receptor is required for normal muscle contraction during physical activity. Conformation of ryanodine receptor to leaky channels by protein kinase A (PKA) hyperphosphorylation, S-nitrosylation, and depletion of the phosphodiesterase (PDE4D3) and the ryanodine receptor stabilizing subunit calstabin 1 (FKBP12) would impair Ca²⁺ signaling and force generation, and decrease exercise capacity. It was shown that S107 (a small molecule) improved exercise capacity by prevention of depletion of calstabin 1 from the RyR1 complex, and reduction of plasma levels of creatine kinase and calpain (Ca²⁺-dependent neutral protease) activity [11].

3.2.1.2 AMPK/PGC1-α Pathway

AMP-activated protein kinase (AMPK) is a key regulator of muscle metabolism and energy turnover. It regulates PGC-1α modification which in turn activates transcription factors involved in mitochondria biogenesis [12]. It was indicated that AMPK activation increases the *PGC-1α* gene expression and directly induces phosphorylation

of PGC1- α [13]. PGC-1 α is a transcription coactivator which activates nuclear respiratory factor (NRF) 1 and 2 that resulted in mitochondrial transcription factor A (mtTFA). It was demonstrated that transcription and replication of mitochondrial DNA are regulated by mtTFA [14]. Several studies showed impairment of training performance after knockout mutation in *PGC1- α* gene, and regulation of the expression of genes related to glucose and lipid metabolism by AMPK-activated sirtuin 1 (SIRT1)-induced PGC1- α deacetylation, revealing the regulatory role of PGC1- α [15]. Moreover, exercise-mediated decrease in acetyltransferase GCN5 (general control non-repressible 5) may increase PGC1- α deacetylation and activity [16].

3.2.1.3 IGF-1/Akt/mTOR/p70s6K Pathway

Increases in muscle mass and protein synthesis were shown to be regulated by mammalian/mechanistic target of rapamycin (mTOR) [17]. IGF-1 induces mTOR activity and increases muscle growth through phosphatidylinositol 3-kinase (PI3K)/Akt pathway [18]. mTOR regulated initiation of the downstream substrates translation including p70s6K (ribosomal protein s6 p70 kinase) and translation elongation protein 4E-BP1 (eukaryotic initiation factor 4E-binding protein1). Since mTOR has a large molecular size, post-exercise evaluation of this protein by western blotting is difficult. Therefore, in human studies, phosphorylation of its downstream substrates was evaluated. In KO mice with mutated mTOR, the significance of this pathway in exercise-induced muscle growth was approved.

In resting muscle, mammalian target of rapamycin complex 1 (mTORC1) was indicated as a key regulator of mitochondrial protein expression. PGC1- α , the transcription factor Yin Yang 1, mTOR, and raptor are mTORC1 components in this regulation.

An in vitro study investigated this mechanism in chronic contractile activity (CCA) and rest. The effect of CCA on the response of myotubes of murine skeletal muscle cell culture was examined in the absence/presence of rapamycin (as an mTORC1 inhibitor). CCA increased cytochrome

oxidase (COX) IV, COX, and mitochondrial transcription factor A (Tfam) activity, but rapamycin did not suppress these effects. Rapamycin alone decreased organelle state 3 respiration, while it increased COX IV and Tfam mitochondrial content. The authors of the study suggested that mTORC1 activity is necessary for mitochondrial function in resting conditions; however, it is not an integral factor for CCA-induced elevation of mitochondrial content [19].

3.2.1.4 Myostatin/Smad Pathway

Skeletal muscle disuse and mechanical unloading would result in muscle loss and atrophy.

Cachexia (muscle atrophy) is one the complications of disabling diseases like cancer, heart failure, renal failure, chronic obstructive pulmonary disease, and AIDS. Inadequate cytokine and inflammatory responses as well as muscle disuse would induce and initiate signaling pathways including myostatin/Smad, IGF1-Akt-mTOR, E3 ligases of the ubiquitin proteasome, and Atrogin-1/MAFbx and MuRF1 which mediate skeletal muscle protein adaptation to disuse and inflammation [20]. Myostatin (growth differentiation factor 8) belongs to transforming growth factor (TGF) β superfamily and is a ligand for activin receptors which regulate downstream Smad proteins [21]. It was shown that inhibition of Smads transcriptional activity would result in muscle hypertrophy [22].

Short and long lived skeletal muscle protein degradation is carried out through ubiquitin-proteasome pathway including E1, E2, and E3 ligases. Muscle Atrophy F-box (MAFbx/atrogin-1) and Muscle-specific RING Finger protein1 (MuRF1) are two main muscle E3 ligases that are controlled by Forkhead box O (FOXO) of transcription factors. Under normal conditions, activation of IGF-1-PI3K-Akt-mTOR pathway leads to Akt inhibition of FOXO which induces muscle growth and suppression of muscle atrophy [18, 20].

It was indicated that carbohydrate intake during aerobic physical activity inhibits the expression of proteolytic genes by skeletal muscle microRNA regulatory action on PI3K-Akt-FOXO1 pathway [23].

3.2.2 Exercise-Induced Adaptation in Muscle Fiber Type

In the skeletal muscle of adult human, slow and fast fiber types and their isoforms type including I/ β (for slow fibers), and IIa, II d/x , and IIb (for fast fibers) were identified. The physiologic (morphological, biochemical, and functional) characteristics of these isoforms including fiber-specific gene expression patterns, strength, contraction speed, and fatigability are different [24, 25]. Aging, exercise, and pathological conditions were shown to modify transcription factors related to the muscle fiber isoform. Gene expression modifications which lead to muscle plasticity were not fully elucidated [26].

Estrogen-related receptor gamma (ERR-gamma) is a nuclear receptor that acts as constitutive transcriptional activator. The role of this receptor in modification of fiber type and oxidative metabolism was indicated in transgenic mice of muscle-specific VP16 ERR-gamma model. The results showed that exercise induced muscle type conversion and increased oxidative capacity (i.e., enhanced mitochondrial biogenesis and enzymatic activity) in this model. Nevertheless, exercise capacity and mitochondrial activity were decreased in mice missing one copy of ERR-gamma. In addition, muscle gene expression profile was shifted toward oxidative fiber (red) type muscle following ERR-gamma elevation. A small molecule agonist for ERR-beta/gamma produced a stimulatory effect on mouse myotubes' mitochondrial activity. The authors of the study concluded that ERR-gamma activation could have therapeutic effects on metabolic diseases with impaired oxidative metabolism and lower content of red type muscle fiber [27].

3.2.3 Exercise-Induced Metabolic Adaptation of Skeletal Muscle

Skeletal muscles are known as a key metabolic organ. In resting adults, 30% of basal metabolic rate is attributed to skeletal muscles [28]. When stimulated by insulin, skeletal muscles markedly contribute to glucose deposition and become the

main organ for glycogen storage. Moreover, physical activity induces insulin-independent muscle glucose uptake. Therefore, skeletal muscles play critical roles in metabolic homeostasis, glycemic control, and prevention and management of metabolic disease. Because of these metabolic roles and the fact that skeletal muscle is the main organ affected by exercise training, human muscle biopsy was used to reveal molecular physiology of exercise. A main impact of exercise on skeletal muscle biochemistry and metabolic function is mediated through modification of mitochondrial biogenesis and function. It was well documented that exercise training induces mitochondrial remodeling (in terms of functional and molecular aspects) of skeletal muscles. Physical activity might result in two-fold increase in mitochondrial density and oxidative phosphorylation capacity [29].

One of the contributors to exercise-induced metabolic changes in muscles is the increase in fatty acid (FA) delivery and oxidation. It is well known that PPAR δ [30], PGC-1 α [31] and ERR-gamma [27] are involved in exercise-induced FA oxidation. It was thought that FA may enter the cell by simple diffusion and induce changes in biological machinery, mitochondrial enzymes and biogenesis [32]. However, recent findings showed that the fatty acid transporter CD36 might be a key regulator of FA membrane transport that modulates fatty acid oxidation in resting and active conditions [33].

3.2.3.1 Endurance Exercise Adaptation

The beneficial effects of aerobic endurance exercise on enhancement of cell oxidative capacity were shown to be mediated through enhancement of mitochondrial oxidative enzymes content [34] and cellular insulin sensitivity in geriatrics [35].

It was demonstrated that exercise could improve obesity and insulin resistance induced by cafeteria (low fat) diet. Swimming exercise ameliorated cafeteria-diet-induced decrease in insulin-stimulated glucose transport in red gastrocnemius muscle of the perfused hind limb of rats [36].

3.2.3.2 Resistance Exercise Adaptation

Chronic resistance exercise enhances mitochondrial respiration capacity of skeletal muscle both

quantitatively and qualitatively by modest increases in the mitochondrial gene expression and proteins content in young adults [37]. Resistance exercise also ameliorates age-induced skeletal muscle loss by restoration of the decline in synthesis rates of proteins and transcription of myosin heavy-chain gene of muscle [38].

Implementing resistance exercise upregulates the IGF-1-PI3-Akt-mTOR pathway gene expression in the muscle which increases the synthesis of muscle proteins and muscle mass [20]. Exercise and muscle loading increase both autocrine and endocrine IGF-1 levels [39].

Degradation pathways are essential for maintenance of skeletal muscles. Chaperone-assisted selective autophagy (CASA) is one of these pathways, and it increases after the muscle undergoes tension. After acute bout of strenuous resistance exercise, CASA induction was found to act as an adaptation mechanism for degradation of damaged proteins of muscle cytoskeleton. In addition, repeating resistance exercise bouts for 4 weeks increased the expression of CASA components [40].

3.2.3.3 Combined Exercise Adaptation

Combined exercise protocols of training such as high-intensity interval training (HIIT) or sprint interval training (SIT) were also demonstrated to possess the advantages of both endurance (aerobic) and resistance exercise, despite the fact that the intensity of resistance and endurance components of combined exercises is lower than that of each individual training [41]. In young healthy men and women, the effect of 6-week SIT was compared to that of endurance training. The results showed comparable improvement in muscle markers of oxidative capacity and mitochondrial enzymes, including pyruvate dehydrogenase E1 α protein content, 3-hydroxyacyl CoA dehydrogenase maximal activity, and PGC-1 α , after both types of training. In addition, after training, muscle consumption of phosphocreatine and glycogen decreased, while the rate of whole body lipid oxidation increased [42]. However, the intensity of training is an important determining factor of adaptation process as it was indicated

that low-intensity training may restrict the mitochondrial response [43].

It was well documented that HIIT elevates the levels of proteins related to lactate transport, glycolysis, and glycogenesis which consequently lead to increment of muscle glycolytic capacity. Hypoxia-inducible factor-1 (Hif-1) α is one of the major regulators of expression of proteins that contribute to anaerobic metabolism. Six-week HIIT was shown to increase the glycolytic capacity in gastrocnemius muscles. The authors of the study concluded that Hif-1 α acts as a main regulator of muscle metabolic adaptation after HIIT [44].

The evaluation the effect of 12 weeks of HIIT exercise in young adults indicated that increases in VO₂ peak and citrate synthase enzyme activity in the muscle were comparable to those induced by lower-intensity training following longer duration [45].

Transcriptional coactivators and corepressors are important elements in mitochondrial biogenesis modulation in skeletal muscles. Three weeks of high-intensity interval training in human volunteers decreased the levels of p107 protein which has a reciprocal correlation with mitochondrial oxidative phosphorylation [46].

The effect of intense intermittent cycle training on signaling pathways related to mitochondrial biogenesis was evaluated before and after (immediately and after 3-h recovery) collecting vastus lateralis biopsies from healthy subjects. Exercise induced immediate increases in phosphorylation of p38 mitogen-activated protein kinase (MAPK) and AMPK (α 1 and α 2 subunits) and later (after 3 h of recovery) elevation of PGC-1 α mRNA. However, the level of PGC-1 α protein was not changed. While the protein kinase B/Akt (Thr-308 and Ser-473) phosphorylation was decreased, results showed no changes in hypertrophy-related downstream targets (p70 ribosomal S6 kinase and 4E binding protein 1). These findings approved the role of AMPK and p38 MAPK in PGC-1 α regulation in low-volume intense interval exercise-induced mitochondrial changes for effective metabolic remodeling and fatty acid/glucose oxidation [47].

The adaptive muscle response (i.e., mitochondrial capacity) to an acute bout of HIT was

evaluated in vastus lateralis muscle biopsies of healthy subjects. Samples were obtained before and after (immediately and after 3- and 24-hour recovery) exercise. In resting condition, PGC-1 α was detected in cell lysate. AMPK and p38 MAPK were activated by training, and the expression of mitochondrial genes' mRNA and nuclear PGC-1 α protein was increased. Moreover, 24 h after exercise, mitochondrial changes including increases in enzymatic activity and protein content were detected which reflect the key role of PGC-1 α in muscle adaptation to HIT [48].

Analyzing the biopsies of skeletal muscle collected from untrained healthy males before and after a single bout of high-intensity exercise showed training-induced alterations in protein phosphorylation. Results showed 1004 unique phosphosites on 562 proteins that were regulated by exercise training. Among them, there were substrates of known kinases (i.e., mTOR, MAPK, PKA, AMPK, and calmodulin-dependent protein kinase (CaMK)), though most of them were unknown elements of exercise signaling. Moreover, AMPK-dependent A-Kinase Anchoring Protein 1 (AKAP1) phosphorylation showed to have an important role in exercise-induced mitochondrial alterations [49].

Miyamoto-Mikami et al. studied changes in skeletal muscle gene expression in young men after 6-week high-intensity intermittent exercise training. Induction of mitochondrial biogenesis after HIIT exercise could be attributed to changes in gene expression which was consistent with previous reports. Gene ontology analysis showed upregulation of exercise-related genes including *CARNS1*, *FGF6*, *MYLK4*, *PGK1*, *PPP1R3C*, and *SGK1*, as well as encoded protein genes *CARNS1*, *MYLK4*, *PPP1R3C*, and *SGK1* [50]. After HIIT exercise, signaling pathways related to mitochondrial biogenesis (such as PPAR γ Coactivator 1 Alpha protein as a signaling molecule) [47, 48, 50] and mitochondrial enzymes (e.g., citrate synthase) were activated [42, 48, 50, 51].

3.2.3.4 Comparison of the Effects of Exercise Modalities on Adaptation

Exercise-induced mitochondrial and metabolic changes were shown to be affected by type (endurance and resistance), intensity, and duration of training bouts. Resistance training modulates myogenesis and ratio of muscle protein synthesis to degradation, while endurance training improves angiogenesis, biogenesis of mitochondria, and metabolism of fatty acid metabolism. Moreover, it was demonstrated that HIIT would lead to a combination of endurance and resistance training effects. So HIIT results indicated improved oxidative and glycolytic capacity, mitochondrial enzyme activities, intramuscular triglyceride and glycogen storage levels, and angiogenesis.

However, more investigation should be undertaken to reveal different adaptation changes induced by various types of training. Wang et al. investigated the influence of endurance and resistance exercise on mitochondrial components and their related signal transductions. In a randomized crossover study, healthy individuals trained only under endurance exercise program or endurance training followed by resistance training (ER) and the expression levels of mRNA of related mitochondrial genes were evaluated in muscle biopsies which sampled pre- and post-(1- and 3-h post-cycling) exercise training. The results showed elevation in mRNA of pyruvate dehydrogenase kinase-4, PGC-1 α , and PGC-1-related coactivator (PRC) after both endurance and endurance plus resistance training; however, the mRNA expression levels of such genes were higher in ER (endurance + resistance) group. In addition, synthesis of ribosomal S6 kinase 1, eukaryotic elongation factor 2, and mTOR increased in post-training samples of the ER group. Moreover, the expression of genes related to mTOR signaling (i.e., cMyc and Rheb) increased after ER exercise. Additionally, 1-h post-cycling samples obtained from both endurance exercise and RT groups showed increased levels of acetyl-CoA carboxylase, AMP-activated protein kinase, and Akt protein phosphorylation. These findings approved the beneficial effects of combined endurance and resistance training on

mTOR-mediated mitochondrial biogenesis and adaptive increase with respect to oxidative capacity [52].

In Robinson et al.'s study, effects of different exercise modalities on old and young participants were investigated. It was postulated that aging-induced reduction of mitochondrial content is correlated with a decline in cardiorespiratory fitness in the elderly [53]. In addition, impaired mitochondrial ATP production in resting condition may facilitate progression of age-induced insulin resistance [54]. In this study, the correlation between insulin-resistant states and reduced mitochondrial oxidative capacity of skeletal muscle, and the role of exercise was examined in young and old subjects in resting and fasting conditions. At the beginning of the study, mitochondrial respiration of old subjects was lower compared to young individuals. Maximal mitochondrial oxygen consumption of both old and young individuals was increased after 12 weeks of HIIT training. However, this marker increased only in young subjects after combined training (CT), but resistance training (RT) had no effect on it in both groups. There were no differences in mitochondria intrinsic markers (reactive oxygen species (ROS) production, and coupling efficiency) between the two groups after HIIT, RT, and CT programs. Decrement of mitochondrial respiratory capacity in aged individuals might be due to alterations in mitochondrial protein levels which could be improved by physical training. The number of normalized mtDNA copy in old subjects was lower than young participants, and HIIT and RT significantly improved mtDNA; however, the increasing effect of CT on mtDNA was nonsignificant. HIIT was shown to combat age-induced downregulation of the expression of genes related to insulin signaling, mitochondrial function, and muscle hypertrophy. Among different exercise regimes, HIIT had better impacts on these signaling pathways, and the effects of HIIT were more marked in old individuals compared to young people. In old adults, after HIIT, the expression of 22 genes such as those related to regulation of translation (ribosomal *MT-RNR1* and 2) and mitochondrial tRNA transferase for leucine (*MT-TL1*), valine (*MT-TV*), glycine (*MT-TG*),

methionine (*MT-TG*), and arginine (*MT-TR*) was increased. The impact of RT and CT on gene expression was less than that of HIIT. These findings indicated that exercise's beneficial effects are associated with the type (or modality) of training as well as genetic and epigenetic factors. Taken together, in both old and young subjects, HIIT improved aerobic capacity markers (mitochondrial respiration and insulin sensitivity) but had no significant effect on anaerobic capacity. RT and CT exercise had a remarkable impact on muscle mass and strength elevation in both age groups, and it also enhanced performance and muscle metabolic storage by increasing non-oxidative glucose disposition index [2].

HIF-1 is a key factor in modulation of cell response to hypoxia and causes its effects via increasing the expression of genes regulated to oxygen delivery or glucose metabolism. Therefore, HIF-1 significantly contributes to adaptation to exercise-induced hypoxia. However, there is not enough evidence on changes in HIF-1 and its target genes after chronic exercise training. In addition, acute exercise-induced elevation in HIF-1 may be suppressed following long-term training. The negative regulatory effects of chronic exercise on HIF-1 might have beneficial impacts on muscle metabolism. HIF-1 inactivates pyruvate dehydrogenase complex by increasing pyruvate dehydrogenase kinase 1 and reducing the flux of pyruvate into the mitochondria resulting in endurance performance decline. Therefore, deletion of HIF-1 might lead to better training adaptation in muscles [55]. An epigenetic corepressor of HIF-1 is sirtuin 6 (SIRT6) which is a histone-3 lysine-9 deacetylase that could improve mitochondrial activity by targeting the glycolytic genes [56]. Based on such knowledge, the effect of long-term training on negative regulation of HIF-1 and related mechanisms were studied in two human investigations; a longitudinal before/after study on the effects of six-week training in individuals and a cross-sectional study on elite athletes and moderately trained subjects. The results showed a remarkable elevation of muscle HIF suppressors in elite athletes following endurance training protocols. The authors suggested that lower phosphoinositide-dependent protein

kinase-1 level in those athletes is a consequence of high activity of prolyl hydroxylase domain-containing protein 2, as its expression is probably regulated by its tricarboxylic acid cycle substrate, hypoxia, and HIF-1. In addition, the levels of HIF transcriptional factors inhibitors including factor-inhibiting HIF (FIH) and SIRT6 were increased in elite athletes [55]. It was shown that a single bout of running in sedentary individuals induced five-fold increase in p300/CBC which is a coactivator of HIF but decreased SIRT6 as an HIF inhibitor. However, in trained individuals, p300/CBC decreased, approving the hypothesis of HIF negative regulation in chronic exercise [57]. Therefore, the early phase of muscle adaptation to exercise might be quite different from the late phase. In early phase, HIF activation is necessary for elevation of vascular endothelial growth factor (VEGF) level and angiogenesis in muscle [58]. It seems that increases in the number of muscle capillaries occur before mitochondrial enzymatic changes and in muscles oxidative capacity increases supported by upregulation of pyruvate dehydrogenase complex activity in elite subjects [59].

3.3 Cardiovascular Adaptations

Cardiovascular benefits of exercise training and physical activity were emphasized in the literature, but molecular mechanisms and signal transduction related to those effects are poorly elaborated [60]. The effect of exercise on different human physiological systems and even within each system (e.g., cardiovascular system) may vary based on exercise modality (e.g., endurance vs resistance), training intensity, and duration and repetition of bouts [3]. The studies discussed below showed the therapeutic and preventive effects of different exercise modalities on cardiovascular diseases.

Pacemaker activity of SA (sinoatrial) node is mediated by f-channels (hyperpolarization-activated, nonselective cation current) which are regulated by cAMP. The findings of a study using mutant mice with hyperpolarization-activated cyclic nucleotide-activated (HCN) isoform channel (hHCN4-573X) showed that cAMP regulation is responsible for the determination of basal and

maximal heart rate, and during exercise, other factors may have affect heart rate [61]. In addition, sympathetic stimulation by exercise would increase heart contractility by activation of beta-adrenergic receptors and protein kinase A. It was demonstrated that PKA phosphorylates the cardiomyocyte ryanodine receptor (RyR2) at Ser2808, resulting in more marked Ca^{2+} influx and contraction [62]. It was also demonstrated that chronic exercise could change isoforms of myosin light chain 1 subunit which may lead to alteration of molecular pathways of cardiac myocytes' contractile response. These findings showed that exercise produced force-length relationship changes, increased myocytes sensitivity to Ca^{2+} activation, and increased cardiac power output [63].

The effect of exercise on cardiac myocyte autophagic process was evaluated. The levels of autophagy-related proteins and microtubule-associated protein 1 light chain 3 (LC3)-II expression (autophagy indicator) were examined in left ventricle of control and single-bout-exercised rats. Samples were obtained immediately and 0.5, 1, and 3 h after a 30-min running exercise. In exercise group, there was a time-dependent change in LC3-II as it decreased immediately, increased 1 h after exercise, and returned to rest levels 3 h after exercise. Phosphorylation of AMPK alpha was increased immediately after exercise. In addition, exercise-induced changes in the mTOR phosphorylation (as an autophagy inhibitor) were opposite to LC3-II expression alterations. These findings showed a role for mTOR-mediated pathway in cardiac muscle autophagy changes after training exercise [64]. Myocardial mitochondrial function has a key role in regulation of cellular death and supplying energy. Pathological conditions such as ischemia may induce fragmentation and fission of cardiomyocyte mitochondria which results in mitophagy and cellular death. This hypothesis was questioned by recent investigations showing that suppression of mitochondrial fission may cause cardiac dysfunction. The adaptive role of mitochondrial fission and fragmentation in maintenance of cardiac optimal function during exercise was indicated. It was shown that

beta1-adrenergic receptor-mediated physiological mitochondrial fragmentation during sub-maximal training may improve mitochondrial function. In this type of fragmentation, dynamin-related protein 1 is also activated, but downregulation of mitophagy and maintenance of membrane potential by exercise, induce regulatory mechanisms which maintain normal mitochondrial function and cover energy demand [65]. The effects of ginsenoside Rg3 supplementation and aerobic exercise on mitochondrial function were investigated in rat cardiac muscle. In both groups (one received ginsenoside Rg3 and the other underwent aerobic exercise), cardiac muscle protein levels of PGC-1 α and nuclear factor-E2-related factor 2 (Nrf2) were increased. PGC-1 α activation resulted in elevation of Nrf1 expression and Tfam mRNA levels which led to increases in mitochondrial DNA and enhanced levels of proteins including quinone oxidoreductase 1 (NQO1), superoxide dismutase, catalase, and nicotinamide adenine dinucleotide phosphate. Exercise, similar to ginsenoside Rg3, increased expression of autophagy-related protein 7 (ATG7) and beclin1 as well as conversion of LC3-I to LC3-II. Therefore, both exercise and ginsenoside Rg3 could improve mitochondrial function and remodeling [66].

It was well documented that exercise-induced cardiac hypertrophy due to adaptive physiological changes may lead to higher power output and lower heart fibrosis. The key role of heat shock proteins and their related gene (heat shock transcription factor 1) in this process was indicated. Therefore, some of the protective effects of exercise against cardiovascular diseases might be mediated via increased expression of heat shock transcription factor 1 and synthesis of heat shock proteins [67].

Calcitonin gene-related peptide (CGRP) might act as a vasodilator agent and decrease the infarct size during cardiac ischemia. The effect of single-bout exhausting exercise and chronic aerobic training (8 weeks of swimming) on CGRP mRNA expression in rat cardiac muscle and aortic arch was evaluated. Single-bout exercise had no significant effect on this gene, while chronic exercise increased the expression of CGRP

mRNA in cardiac muscle but had no adaptive impact on aorta [68].

Exercise beneficial effects in coronary heart disease patients were shown to be mediated by increased phosphorylation of endothelial NO synthase (eNOS) via protein kinase Akt pathway. However, in normal mice, this pathway might not act effectively. In this regard, it was indicated that the expressions of platelet endothelial cell adhesion molecule-1, phosphorylated eNOS at Ser1177, protein kinase Akt, phosphorylated Akt at ser473, and eNOS proteins, were not altered after 24 weeks of exercise in healthy mice [60]. The role of NOS1 in exercise-mediated beneficial effect on myocardial contractility was evaluated in mice after 8-week aerobic interval training. In trained mice, cardiac hypertrophy index, VO₂ max, level of NOS1 expression, and nitric oxide content were higher than the control animals. The effect of NOS1 on contractility might be mediated through increment of Ca²⁺ cycling and phosphorylation of phospholamban in cardiac myocytes [69].

One of the common complications of allogeneic hematopoietic stem cell transplantation is post-operative chronic graft-versus-host disease (cGVHD) which leads to morbidity and disability. The beneficial effects of 11 weeks of moderate exercise on cGVHD mice were indicated. In trained animals, cardiomyocyte markers of autophagy including Atg12, phospho-ULK1 (S555), SQSTM1/p62, and LC3BII were increased. Moreover, myocardial glutathione reductase, catalase, and alpha-tubulin contents were higher in exercise group compared to sham group. These results showed that exercise protects against debilitating cardiac disease [70].

The protecting effects of 5 weeks of moderate exercise on cardiomyopathy induced by obesity-associated type-2 diabetes (db/db) were demonstrated. Results of in vivo experiment showed improvement of ejection fraction and fractional shortening in exercised group. In isolated cardiomyocytes of exercise group, velocity of contraction and maximum contraction were increased. In addition, connexin 43 levels and markers of mitochondrial function including mitochondrial biogenesis regulators (Mfn2/Drp-1 levels),

mitochondrial trans-membrane potential and cytochrome c leakage were improved in exercised db/db mice. These findings showed the beneficial effects of moderate exercise on diabetes-induced cardiomyopathy [71].

Cachexia is one of disabling complications of cancer which is characterized by muscle and adipose tissue loss, fatigue and suppressed cardiac function. The molecular mechanism mediating decrease in myocardial function of left ventricular mass and the role of exercise were investigated in a tumor-bearing animal model. Exercise could prevent or modulate inflammatory and oxidative signaling pathways which were activated by inflammatory cytokines and ROS [72]. It was indicated that although exercise could not prevent cancer-induced remodeling in the heart, disorganization of myofibers, interstitial fibrosis, and cardiomyocyte enlargement decreased in trained animal [73].

3.4 Adaptations Observed in Other Physiological Systems

Most of investigations documented the metabolic and biochemical effects of exercise in skeletal muscle, heart, vascular, liver, adipose, endocrine, and brain tissues; however, few studies were done using other tissues such as kidney, lung, pancreas, colon, and immune.

3.4.1 Central Nervous System Adaptations

Neuroendocrine and immune system have direct and indirect critical roles in exercise-induced adaptation response. The central nervous system coordinates neuroendocrine signaling for cardiovascular, respiratory, and metabolic responses to exercise and controls the quality of exercise (initiation, intensity, duration, and termination) [74]. In different models of memory deficit and cognitive disorders, the neuromodulatory effects of exercise on brain molecular pathways were demonstrated. These beneficial effects are increases in

immediate-early gene c-Fos expression in dentate gyrus; enhanced Wnt3 expression; suppressed glycogen synthase kinase 3 expression; increased numbers of bromodeoxyuridine-positive and doublecortin (DCX)-positive cells; increased levels of astrocytes glial fibrillary acidic protein and decreased levels of S100B protein, enhanced BBB integrity; prevention of oxidative stress injury, induction of morphological changes in astrocytes of the stratum radiatum of CA1 area; increased cell proliferation and suppressed apoptosis in dentate gyrus; increased levels of brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) expressions; and enhanced levels of glycogen and normalized expression of monocarboxylate transporter 2 [75].

3.4.2 Nervous System and Skeletal Muscle Cross Talk

Despite the growing evidence on the ameliorating effect of exercise on cognitive, neurodegenerative, and chronic inflammatory disease, the nature of neuroendocrine, immune, and metabolic system cross talk during exercise is not yet fully elucidated. During muscle contraction, myocytes release cytokine and myokines which exert autocrine, paracrine, and endocrine effects in muscle and remote tissues including liver, adipose and brain tissue [76]. Exercise increases serum concentrations of metabolism products including pyruvate, lactate, glycerol, beta-hydroxybutyrate (BOHB), and amino acids (glutamine and alanine) [77]. It was shown that exercise increases the expression of monocarboxylate transporters (MCT 1, 2 and 4) in rat hippocampus and cortex. MCTs transport lactate and BOHB across the blood-brain barrier (BBB). These molecules act as signaling mediators through binding to hydroxycarboxylic acid receptor (HCARs) [78, 79] and improving memory, calcium signaling, neural activity, axonal myelination, and angiogenesis in the brain [80]. BOHB was indicated to decrease binding of histone deacetylase (HDAC 2 and 3) to BDNF promoters which increase hippocampal BDNF level and glutamate release [79].

Lactate receptor, HCAR1, mediates VEGF-A expression and angiogenesis by activating ERK1/2 and PI3K/Akt pathways [78]. Other mediators including irisin, kynurenine and kynurenic acid, cysteine protease cathepsin B (CTSB), IL-6, IL-15, chemokine CXC ligand 1 (CXCL-1) and the leukemia inhibitory factor (LIF), fibroblast growth factor 21 (FGF21), muscle Bmal1, nitric oxide (NO), ROS, and ATP are intra- and extra-skeletal muscle effectors [76].

Cathepsin B is a protease encoded by CTSB gene which is induced by exercise in muscle and other human tissues. It was indicated that CTSB crosses the BBB and might induce transcription of hippocampal DCX and BDNF [81]. In human and rhesus monkeys, treadmill exercise for 4 months increased the CTSB serum concentration which might be correlated with memory improvement [82].

Exercise-induced production of cytokines including IL-6, IL-8, IL-15 mRNA, murine chemokine CXC ligand 1, and leukemia inhibitory factor (LIF) is involved in many metabolic effects of exercise (e.g., insulin release, glucose uptake, and fatty acid mobilization and oxidation) [76, 83]. Elevations of peripheral and central levels of IL-6 cytokine superfamily members and other pro-inflammatory cytokines inhibit BDNF expression [84].

Irisin precursor protein (fibronectin type III domain containing 5 (FNDC5)) secretion by skeletal muscle is increased after exercise training. This myokine is also secreted from other tissues like the brain and acts as a thermoregulator of the nervous system, bone, and especially adipose tissue by elevating energy expenditure. Although in clinical investigations, the role of irisin in beneficial effects of exercise on hippocampus and memory-related brain function was not established, it was indicated that exercise-induced elevation of irisin might mediate differentiation of embryonic stem cells to neural cell and neurogenesis in mice (reviewed in [85]). Nevertheless, it was reported that subject response in terms of myokine (irisin) changes varies based on the exercise modality [86].

A defect in degradation of kynurenine (L-tryptophan metabolite) to kynurenic acid is associated with cognitive disorders such as

depression. It was indicated that exercise increases kynurenine aminotransferase (KATs) expression in the skeletal muscle which leads to enhancement of kynurenine uptake from circulation and its conversion to kynurenic acid. Therefore, the protective effect of exercise against stress-induced disturbance in the brain could be related to the effect of KATs induced via PGC-1 α - and PPAR α/δ - dependent pathway, before the activity [87].

Circadian rhythms control genes or clock-controlled genes (CCG) are detected in almost all cells of mammalian body. Recent studies demonstrated that CCG are not only expressed in the brain but also in other tissues [88]. Therefore, a muscle brain signaling pathway may take part in sleep and circadian rhythm regulation [89]. Bmal1 protein is one of the main regulators of gene transcription related to control of circadian rhythms. Moreover, Bmal1 and other clock genes are candidates for determination of susceptibility to metabolic diseases [90]. Mutations in CCG including CLOCK, Rev-erb alpha and Rev-erb beta, Per1 and Per2, and ROR genes were showed to result in lower exercise-induced oxidative capacities, impaired endurance exercise, and defective muscle contraction and locomotion [91].

Fibroblast growth factor (FGF) superfamily is responsible for regulating several metabolic and developmental signaling pathways including energy balance, glucose and lipid metabolism, angiogenesis, neuroprotection, and behavior [92, 93]. FGF19 and FGF21 are members of this superfamily, and their genes are expressed in various tissues including the liver, muscle, brain, adipose, and pancreas [92]. FGF21 might be considered a myokine involved in cross talk between the brain and muscles [76, 93]. In muscle, it may act as a transcriptional co-regulator for PGC-1 α , or activator of mTORC1 and AKT pathways [94, 95]. The effects of resistance and endurance training on FGF19 and FGF21 serum concentrations in healthy subjects were evaluated. After resistance exercise, the FGF19 level decreased, while endurance training increased plasma glucagon and thereby caused FGF21 elevation [96]. In addition, it was demonstrated that FGF21 increment after

resistance exercise was more pronounced than that observed following HIIT [86].

3.5 Perspectives

Although several experimental and human studies have documented the molecular mechanisms underlying beneficial and preventive effects of exercise, there are still several unanswered questions in this field. Most of the clinical evidence was obtained using muscle biopsy, and there are few findings about the impact of exercise on signaling pathways of other tissues. In addition, the available data on gene expression and signaling peptide production was achieved by computational studies using resting muscle for generating cDNA libraries; thus, more investigations in active muscle are needed. Moreover, according to recent investigations, skeletal muscle might be considered an endocrine metabolic tissue. However, the nature of inter- and intra-organ cross talk of muscle and other tissues needs to be investigated in more detail in future. Therefore, designing multicentered standard clinical trials with special consideration of individual variability and exercise modality and duration seems to be helpful for expansion of our knowledge in this field.

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