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Regulating eEF2 and eEF2K in skeletal muscle by exercise

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What is the topic of this review?

This review summarizes the impacts of exercise training on the translation elongation pathway in skeletal muscle focusing on eEF2 and eEF2K.

What advances does it highlight?

This review highlights mechanisms and factors that profoundly influence the translation elongation pathway and argues that exercise might modulate the response. This review also combines the experimental observations focusing on the regulation of translation elongation during and after exercise. The findings widen our horizon to the notion of mechanisms involved in muscle protein synthesis (MPS) through translation elongation response to exercise training.

Abstract

Skeletal muscle is a flexible and adaptable tissue that strongly responds to exercise training. The skeletal muscle responds to exercise by increasing muscle protein synthesis (MPS) when energy is available. One of protein synthesis's major rate-limiting and critical regulatory steps is the translation elongation pathway. The process of translation elongation in skeletal muscle is highly regulated. It requires elongation factors that are intensely affected by various physiological stimuli such as exercise and the total available energy of cells. Studies have shown that exercise involves the elongation pathway by numerous signaling pathways. Since the elongation pathway, has been far less studied than the other translation steps, its comprehensive prospect and quantitative understanding remain in the dark. This study highlights the current understanding of the effect of exercise training on the translation elongation pathway focusing on the molecular factors affecting the pathway, including Ca^{2+} , AMPK, PKA, mTORC1/P70S6K, MAPKs, hypoxia, and myostatin. We further discussed the mode and volume of exercise training intervention on the translation elongation pathway.

Abbreviations

AC: Adenylate Cyclase

AMPK: AMP-activated protein kinase

cAMP: Cyclic adenosine monophosphate

CaM: Calmodulin

CaMKs: Calcium-calmodulin-dependent protein kinases

CaMKIII: Calmodulin-dependent protein kinase III

ERK: Extracellular signal-regulated kinase

eIFs: Initiation factors

eEFs: Elongation factors

eRFs: Elongation release factors

eEF2: Eukaryotic elongation factor-2

eEF2K: Eukaryotic elongation factor-2 kinase

GDF-8: Growth differentiation factor 8

GPCRs: G protein-coupled receptors

GSK-3: Glycogen synthase kinase-3

HIF-1: Hypoxia-inducible factor 1

JNK: c-Jun N-terminal kinase

MPS: Muscle protein synthesis

MPB: Muscle protein breakdown

mTOR: Mechanistic target of rapamycin

mTORC1: Mechanistic target of rapamycin complex 1

MAPKs: Mitogen-activated protein kinases

MSTN: Myostatin

PKA: cAMP-dependent protein kinase

p90RSK1: p90 ribosomal S6 kinase 1

P70S6K: 70-kDa ribosomal protein S6 kinase 1

RTKs: Tyrosine kinase receptors

S6K1: Ribosomal S6 kinase 1

TGF- β : Transforming growth factor- β

TPR: Tetratricopeptide repeat region

1. Introduction

Skeletal muscle is the most metabolically active tissue and plays a central role in protein metabolism [1]. In muscles, proteins are constantly being synthesized or broken down. The balance between MPS and muscle protein breakdown (MPB) determines the amount of protein in muscle and the rate of muscle mass (hypertrophy). The mechanism of controlling skeletal muscle protein turnover can lead to the recognition or treatment of skeletal muscle diseases. Similarly, it can help athletes who are looking to develop muscle mass. It has been shown that exercise is one of the most powerful modifiers of muscle mass. In response to exercise, MPS increases while MPB also increases or remains constant [2]. When the synthesis exceeds the breakdown ($MPS > MPB$), the positive equilibrium condition leads to an anabolic state, which is the underpinning mechanism for increasing muscle mass. Protein synthesis happens in four steps: initiation, elongation, termination, and ribosome recycling. Ribosomes, along with various proteins such as initiation factors (eIFs), elongation factors (eEFs), and release factors (eRFs), collaborate to facilitate the protein synthesis process [3]. Eukaryotic elongation factor-2 (eEF2) and eukaryotic elongation factor-2 kinase (eEF2K) are two important factors of the elongation pathway [4]. They also play pivotal regulatory roles in the protein synthesis pathway [5, 6]. It has been shown that protein synthesis consumes 30 to 50% of the total available energy of cells, so it is a crucial component of the cell economy [7]. The elongation phase spends a vast amount of energy [8, 9], which is mainly provided via oxidative metabolism. Translation elongation is an elaborate process that uses around four ATPs (ATP and GTP) through the elongation step [5, 7, 10]. Hence, it is known as a substantial energy-consuming process. As a result, it could be stemmed while cells' energy demands surpass the supply. Exercise may initiate protein synthesis and elongation pathways, ultimately leading to the maintenance or increase of skeletal muscle mass. However, the process is not fully comprehended due to the lack of a comprehensive and practical perspective of the effects of exercise training on

translation elongation pathways. Furthermore, when the demanding energy is available to cells, activation of the exercise-related elongation pathway appears to be limited.

The present review aims to provide an overview of studies investigating the effect of exercise on the translation elongation pathway. We then discussed new insights into the regulatory factors involved in the pathway. Furthermore, we concentrate on the effects of various exercise training on elongation factors (eEF2K and eEF2), as well as the mechanisms by which the pathway is inhibited or activated.

2. The eEF2K and eEF2 signaling overview

The translation elongation step of protein synthesis is predominantly regulated by eEF2, a 93-95.2 kDa-monomeric protein [5, 7] (nascent chain translocation from the ribosomal A-site to the P-site) [5, 7, 11-15]. When eEF2 is phosphorylated at Thr56, it becomes inactive because it can no longer bind to ribosomes.[5, 7, 16-23], As a result, the elongation rate is diminished [17-19, 24]. Thr56 is the main physiological phosphorylation site of eEF2. eEF2K is the sole protein kinase that can phosphorylate eEF2 at the site of Thr56 [16, 18, 25-27].

eEF2K is an unusual calcium-calmodulin-dependent protein kinase with a mass of about 95–103 kDa [12, 28], which belongs to a small group of the atypical alpha kinase family [21, 29]. The eEF2K is the upstream factor of eEF2 and is a significant factor that controls elongation. Like other protein kinases (PKs), it phosphorylates target proteins at Ser, Thr, or Tyr residues [30]. It regulates the activation or inactivation of eEF2 via controlling phosphorylation [18, 19, 21, 23, 24, 29]. eEF2K includes an N-terminal catalytic domain (calmodulin-binding domain), a C-terminal region (eEF2-binding domain), a TPR-like alpha-helical region and a linker including multiple regulatory phosphorylation sites [31]. In addition to Ca²⁺/CaM, eEF2K is

regulated by phosphorylation [7]. Depending on the site (see table 1 and figure 1), it could be inhibited or activated. eEF2K is also auto-phosphorylated at specific sites like Thr348, Ser445, and Ser500 [7, 32]. It is well recognized that eEF2K is regulated by various stimuli [15], divided into two categories, elongation pathway activators or inhibitors.

Table 1: The Summary of phosphorylation sites of eEF2K in humans.

Site	Signaling pathway	Source and/or reference
Ser61	Ca ²⁺ /CaM	[32]
Ser66	Ca ²⁺ /CaM	[32]
Ser70	mTORC1	[31]
	GSK3	[33]
Ser78	mTORC1	[19, 34, 35] [33]
	Ca ²⁺ /CaM	[32]
	AMPK	[35]
Ser348	Ca ²⁺ /CaM	[32]
Thr348	Ca ²⁺ /CaM	[32, 36, 37]
Thr353	Ca ²⁺ /CaM	[32, 36]
Ser353	Ca ²⁺ /CaM	[32]
Ser358	MEK/ERK	[31]

Ser359	MEK/ERK	[31, 38]
	mTORC1	[38] [39]
	SAPK4	[40]
Ser366	MEK/ERK	[35] [34]
	mTORC1	[34] [35]
	Ca ²⁺ /CaM	[32]
Ser377	MEK/ERK	[41]
Ser392	mTORC1	[31]
	MAPK	[33]
Ser396	mTORC1	[41] [31] [9]
	MAPK	[33]
Ser398	AMPK	[9] [33]
Ser445	Ca ²⁺ /CaM	[32] [36]
Ser470	mTORC1	[31] [33]
Ser474	Ca ²⁺ /CaM	[32] [36]
Ser491	AMPK	[25] [31, 33]
	Ca ²⁺ /CaM	[32]
Ser492	AMPK	[25]
Ser499	PKA	[42]
Ser500	PKA	[12] [42] [33]
	Ca ²⁺ /CaM	[36]

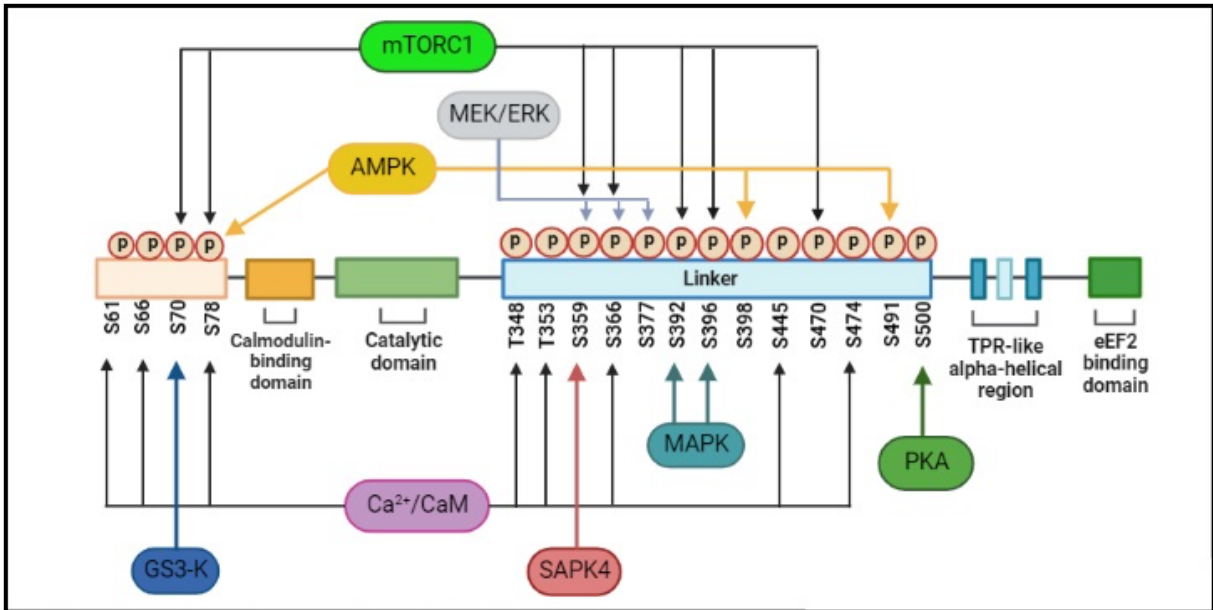


Figure 1: Schematic outlines of phosphorylation sites of eEF2K and the primary functional features of the molecule in humans.

3. Exercise regulates elongation pathway through upstream signaling factors

Exercise can dramatically alter the overall rate of protein synthesis by affecting the cellular pathways; one such example is the translation elongation pathway. Studies have shown that many factors control the translation elongation pathway, such as Ca^{2+} [43-45], AMPK [9, 46-48], mTORC1 [49] and PKA [42, 50, 51] can be altered in response to exercise [49, 52-55]. In the following, these upstream signaling factors will be specifically introduced. We divided these factors into two categories, elongation pathway activators and inhibitors.

3.1. The Elongation pathway activators

3.1.1. mTORC1 / P70S6K

There is solid evidence that mechanistic target of rapamycin (mTOR) signaling acts as a prominent cell size regulator [56, 57]. It is also associated with cell transformation [58, 59] and

the hypertrophy process [60]. mTOR is a large (289 kDa) serine/threonine kinase [61] that consists of two separate messaging complexes named mammalian target of rapamycin complex 1 (mTORC1) and 2 (mTORC2). Although both mTORCs participate in growth control, the mTORC1 is far more important in terms of exercise training, muscle protein synthesis, and hypertrophy [62, 63]. Rapamycin can only suppress mTORC1 [64]. Previous studies have revealed that mTOR inhibition leads to a ~60% decrease in protein synthesis [65, 66]. In addition, rapamycin-inactivation of the mTOR signaling pathway reduces muscle protein synthesis and hypertrophy, even up to 95% [60]. mTORC1 is a dominant regulator protein kinase that has two main downstream targets, the 70-kDa ribosomal protein S6 kinase 1; P70S6K [67] and the eukaryotic initiation factor 4E-binding protein 1; 4E-BP1 [68]. mTORC1 activates P70S6K, which is a positive regulator of protein translation [69]. It poses a significant role in regulating the translation elongation process [16]. P70S6K is the upstream factor of eEF2K. Under anabolic conditions, eEF2K is phosphorylated (Ser366) and inactivated by p70S6k at low Ca^{2+} concentrations [34]. This phosphorylation attenuates the sensitivity of eEF2K to being activated by Ca^{2+} /CaM [31]. In consequence, eEF2K would be unable to repress eEF2, therefore the elongation pathway being activated. Furthermore, mTORC1 has the ability to directly phosphorylate eEF2K, result in (Ser78) eEF2K inactivation [35]. Since this site is located near the CaM-binding site (see fig. 2), it could negatively affect CaM binding to eEF2K [31]. Taken together, mTORC1 is one of the most important regulators of eEF2 and eEF2K [70].

3.1.2. MAPKs:

Mitogen-activated protein kinases (MAPKs), a family of serine-threonine kinases, act as a major regulator of gene transcription, development, metabolism, and apoptosis in skeletal muscle [71, 72]. They mediate extracellular signals for cellular responses. The stimulation of tyrosine kinase receptors (RTKs) and G protein-coupled receptors (GPCRs) can activate

MAPKs [72]. The MAPK family consists of three crucial sub-categories: 1) extracellular signal-regulated kinase 1/2 (ERK1/2); 2) p38 MAPK; 3) c-Jun N-terminal kinase; JNK (also known as stress-activated protein kinase or SAPK), which are stimulated by cytokines, growth factors, and cellular stress [73, 74]. Each of these kinases exists in several isoforms, including ERK1 to ERK8 and JNK1 to JNK3 [75-77]. Concrete evidence has reported that MAPKs or their downstream effectors could instantly down-regulate the eEF2K. [40, 41]. It has been firmly claimed by previous studies that ERK/TSC2 has an influential role in activating mTORC1 signaling [78]. Consistent with these results, Winter et al. showed that both ERK and AKT can equally stimulate mTORC1 and mTORC2 signaling through TSC2 phosphorylation and inhibition [79]. Consequently, it could possibly be implied that inhibiting eEF2K, is a mTORC1–dependent pathway. In addition, Wang et al. (2001) showed that p90 ribosomal S6 kinase 1 (p90^{RSK1}) is activated by ERK, resulting in eEF2K phosphorylation and inhibition at the Ser366 site in embryonic stem cells (ES) [34]. In another study, Wang et al. (2014) showed that in cancer cell lines, ERK directly phosphorylates eEF2K at Ser359, which is independent of mTORC1 [31]. Knebel et al. (2001) investigated the effect of SAPK/p38 (one of the MAPKs family members) on the elongation pathway. They showed that SAPK/p38 can suppress eEF2K [40]. It has been demonstrated that p38 MAPK signaling leads to phosphorylation and switches off eEF2K [80].

Taken together, studies show that eEF2K is phosphorylated and inhibited by MAPKs at different sites. As a result, eEF2 and the elongation pathway are activated.

3.2. The Elongation pathway Inhibitors

3.2.1. Ca²⁺ – calmodulin

Calcium ions (Ca^{2+}) are vital components of cellular signaling. In the cellular environment, Ca^{2+} binds to calmodulin (CaM) and forms the Ca^{2+} /CaM complex [81]. This complex can eventually form the catalytic domain of calcium-calmodulin-dependent protein kinases; CaMKs [81], that are critical in regulating calcium signaling in eukaryotic cells [82]. eEF2K is a Ca^{2+} /calmodulin-dependent kinase, also known as calmodulin-dependent protein kinase III (CAMKIII), which means that the eEF2K activity is dependent on calcium ions and calmodulin [32]. Therefore, changing the concentration of CaM influences the eEF2K in/activation. Studies have shown that CaM is essential for the effective activity of eEF2K [83]. CaM binds to the C-terminal regulatory region of eEF2K [84, 85]. Redpath et al. showed that eEF2K autophosphorylation increased its activity by 2 to 3 fold [50]. CaM makes eEF2K autophosphorylation at multiple sites [32, 36], see table 1. The eEF2K phosphorylation by CaM at these sites activates eEF2K resulting in eEF2 phosphorylation and inhibition of the elongation pathway.

Despite there being abundant phosphorylation sites, the Ser78 site appears to be more important, since along with CaM, the mTORC1 and AMPK pathways also phosphorylate this site [34, 35]. The Ser78 site is closely located to the CaM-binding zone in eEF2 kinase, hence, phosphorylation could disrupt eEF2 kinase binding to CaM [35].

3.2.2. AMPK

AMP-activated protein kinase (AMPK), expressed in all eukaryotic cells, is firmly involved in cellular energy homeostasis and metabolism [86-88]. Metabolic and cellular stressors such as skeletal muscle contraction [89, 90], hypoxia [91], nutrient deprivation [92], oxidative stress [93, 94] cause a rise in the AMP:ATP ratio and activate AMPK [25]. AMPK activation results in, the regulation of numerous signaling pathways, which are associated with maintaining homeostasis and establishing metabolic adaptability. It decreases energy consumption and

increases energy supply via phosphorylating a wide range of target proteins [9]. One of these pivotal signaling pathways is the translation elongation pathway. AMPK phosphorylates and activates eEF2K through mTORC1-dependent and mTORC1-independent pathways. In the case of the mTORC1-dependent pathway, it was shown that AMPK represses mTORC1 signaling and protein synthesis [95-97]. AMPK phosphorylates TSC2 and Raptor which in turns leads to a drop in the activity of p70S6K which phosphorylates and inactivates eEF2K [25, 98, 99]. When it comes to mTORC1-independent pathways, AMPK activates eEF2K through two main mechanisms. Firstly, it increases cytosolic $[Ca^{2+}]$ and subsequently activates eEF2K via calmodulin. Secondly, it causes to direct phosphorylation-activation of eEF2K by AMPK at Ser491/Ser492 [25] and Ser-398 residues [9].

3.2.3. PKA

Cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA) is one of the well-understood protein kinases. Every stimulus that increases intracellular cAMP (For example, increasing the activity of adenylate cyclase (AC) enzyme) activates PKA [100]. Studies have introduced PKA as a negative regulator of cell proliferation and protein synthesis [101, 102], which can phosphorylate eEF2K [12, 28], partially independent of Ca^{2+} /calmodulin [12, 42]. Redpath et al. showed that in the absence of calcium, PKA brings about eEF2K phosphorylation and activation [12]. In this regard, Johanns et al. showed that PKA can phosphorylate and activate eEF2K under low calcium concentration conditions, even though phosphorylation of Ser500 by PKA increases Ca^{2+} -independent eEF2K activity in genetically modified mouse embryo fibroblasts (MEFs) [25]. Studies have also shown that PKA activating factors like β -adrenergic agonists and forskolin boost the cellular levels of eEF2 phosphorylation [51, 103, 104].

3.2.4. Myostatin:

Myostatin (MSTN), also called GDF-8 (Growth Differentiation Factor 8), is released by myocytes, which are known to be a negative regulator of protein synthesis and muscle cell proliferation [105]. This protein, which is primarily expressed in muscle [106, 107], is a member of the transforming growth factor- β (TGF- β) family [108]. Inhibition or inactivation of myostatin causes MPB and muscle hypertrophy; its activation leads to MPB impairment and eventual muscle atrophy [109-111]. Studies have shown that the inhibitory effects of myostatin on MPB are mediated through the AKT-mTOR signaling pathway [109, 112, 113]. In this regard, Deng et al. showed that myostatin activation inhibited the mTOR and eEF2K-eEF2 signaling pathways and subsequently repressed protein synthesis.

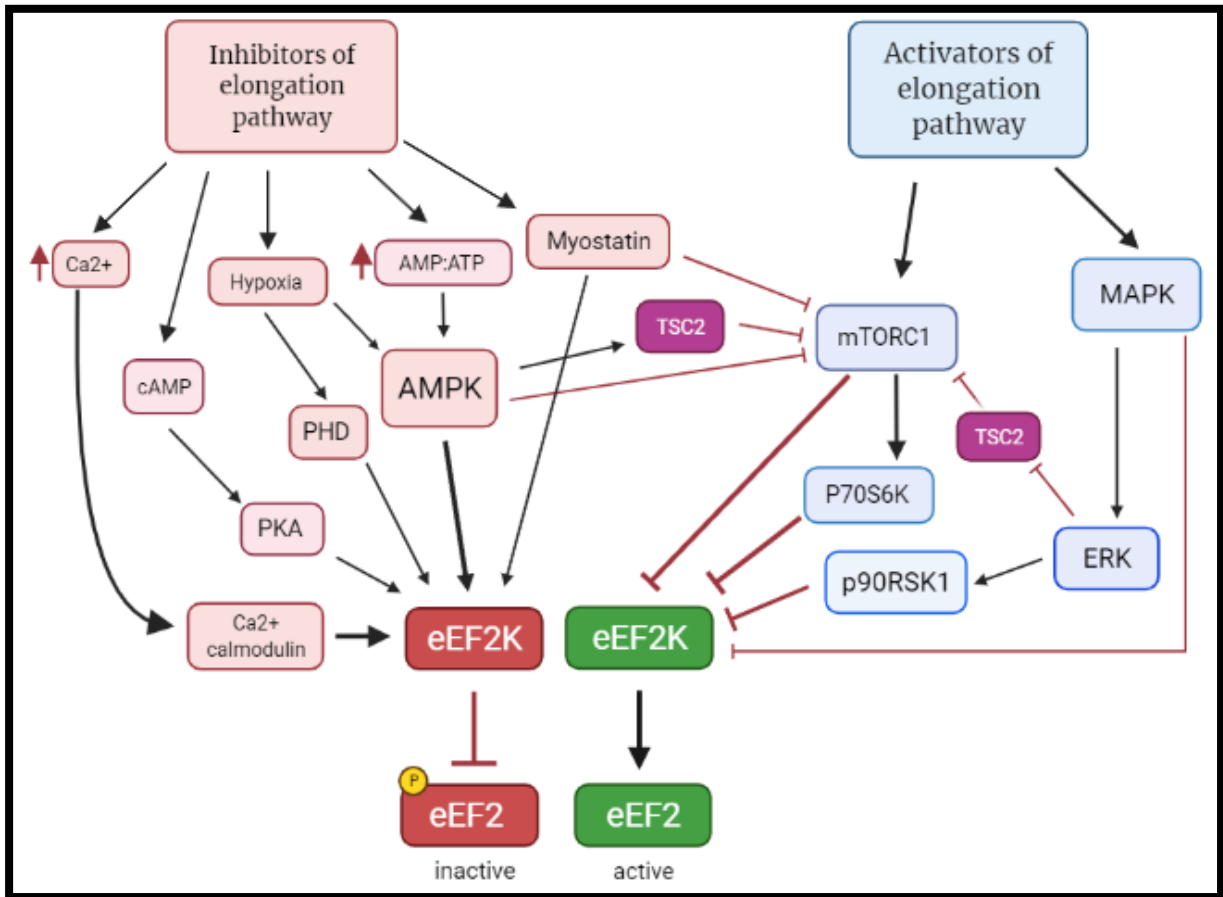


Figure 2: Schematic diagram of possible regulation pathways of translation elongation. The figure contains two activator and inhibitor factors that can activate and inactivate the elongation pathway respectively. The red-color polygons represent inhibitory factors, whereas the blue-color polygons indicate stimulatory factors. Inhibitory factors phosphorylate and activate eEF2K. Then eEF2K phosphorylates and inhibits eEF2 and the elongation pathway will be deactivated. Stimulatory factors phosphorylate and inactive eEF2K. Subsequently, eEF2 is activated and the elongation pathway is also activated. Red T-bars: inhibitory signals, black arrows: stimulatory signals.

4. The response of the elongation pathway to different exercise modes

Here, we divided the studies into two categories: resistance training and endurance training.

We also examine the changing rate of protein synthesis during and after exercise.

Table 2. The Summary of studies investigating eEF2 and eEF2K responses to the resistance exercise in skeletal muscles.

Study	Type of exercise	Results
Rose et al [90]	Electrical contraction	1) Ca ²⁺ /CaM-eEF2K signaling is a stronger factor for eEF2K activity Compared with AMPK 2) eEF2K inhibition, partially (30–40%) prevents inhibition of protein synthesis during resistance training.
Liu et al [114]	high-frequency electrical stimulation	1) one minute after the onset of contraction, eEF2 phosphorylation increased rapidly up to 2 fold and remains high during contraction. 2) eEF2 phosphorylation probably is the main mechanism for MPS Suppression
Macesich [115]	3 sets of leg extensions at a 10-repetition maximum resistance until failure	1) eEF2 phosphorylation was positively correlated with AMPK activity 2) eEF2 phosphorylation increased sharply with the onset of activity and decreased dramatically after activity.
Dreyer et al [116]	Resistance: (10 ×10 repetitions of leg extension exercises on a Cybex leg extension machine set to 70% of 1RM)	1) eEF2 phosphorylation increased during exercise and reduced at 1 and 2 h post-exercise. 2) Increasing mTOR and S6K1 post-exercise overcomes the inhibition of eEF2 by AMPK.

Miranda et al. [117]	Electrical isometric contraction	<p>1) Within 1 minute of the onset of contraction, eEF2 phosphorylation increased rapidly by about tenfold</p> <p>2) There is no correlation between increased eEF2 phosphorylation and AMPK activation.</p>
Edman et al [118]	Resistance: (heavy leg press exercise)	<p>1) At rest, phosphorylation of eEF2 at Thr⁵⁶ was 128% higher in type II than type I fibers</p> <p>2) S6K1 and eEF2 proteins were ~50% higher in type II than in type I fibers</p> <p>2) Resistance exercise led to a 53% reduction in eEF2 phosphorylation in both fiber types.</p>
Ahtiainen et al [119]	Resistance: with leg press device & Endurance: 50-min walking with the extra load on a treadmill	eEF2 phosphorylation decreased following resistance exercise but no changes following endurance exercise
Dreyer et al [120]	high-intensity leg resistance exercise	eEF2 phosphorylation decreased at 1 and 2 h post-exercise
West et al. [121]	Resistance: Electrical contraction	eEF2 phosphorylation decreased in the post-exercise period by mTORC1-independent pathways
Lysenko et al. [122]	Resistance: leg presses with moderate	<p>1) Phosphorylation of eEF2 significantly decreased after high-load resistance exercises</p> <p>2) Following high-load resistance training, phosphorylation of ERK1/2 Leads to a decrease in eEF2 phosphorylation</p>

	load (65% 1RM) & high load (85% 1RM)	
Burd et al. [123]	Resistance: leg extension exercise at different exercise loads	eEF2 activity significantly increased following low-load resistance training compared to high-load resistance training.
Fujita et al. [124]	Resistance: bilateral leg extension exercise	Following resistance exercise combined with blood flow restriction eEF2 phosphorylation significantly decreased
Tannerstedt et al. [125]	Resistance: maximal lengthening contractions with one leg	Phosphorylation of eEF2 was attenuated 20–45% in type II fibers during recovery
Pugh et al. [126]	Resistance: leg extensions, 70% 1RM & Endurance: HIIT at 90% HRmax	HIIT immediately after resistance training did not alter the eEF2
Apró et al [127]	high-intensity and high-volume resistance exercise & 30	eEF2 phosphorylation was reduced ~ by 70% during recovery in both groups: (high-volume resistance) and (high-volume resistance followed by 30 min of cycling)

	min of cycling	
Apró et al. [128]	Endurance: high-intensity interval cycling & Resistance: leg-press exercise	1) eEF2 phosphorylation increased by about 95% immediately after endurance training 2) during the post-exercise recovery period, phosphorylation of eEF2 was reduced by a similar amount of 55% in both groups (interval cycling followed by resistance exercise & resistance exercise only)

*(in all studies eEF2K phosphorylates eEF2 at Thr56).

5. Resistance exercise and control of eEF2K and eEF2

Skeletal muscles have tremendous potential for plasticity, which enables them to respond and adapt to changes in mechanical stress. Resistance training as a common type of exercise can lead to mechanical stress and result in altered cellular and molecular responses in skeletal muscles. In the following, we will discuss the elongation response to resistance exercise. Mechanical stress from acute resistance exercise evidently evokes MPS through specific pathways. The mTORC1 pathway and its certain downstream intermediates, such as eEF2K and eEF2, [120, 129-134] [60, 135], are one of them. Since these crucial components (mTORC1, eEF2, and eEF2K) are critical regulators of MPS, the rate of protein synthesis in response to resistance is to some extent dependent on exercise in response to elongation.

5.1. During Resistance Exercise

A large number of studies confirm that MPS is suppressed during resistance exercise [116, 130, 136]. In addition, studies show that the elongation pathway turns into an inactive state with the onset of resistance exercise and remains unchanged until the end of the exercise [90,

114-116]. Liu et al. investigated the rat skeletal muscle and reported that eEF2 phosphorylation increased up to 2-fold immediately with the onset of contraction. The rise remained high during the contraction. This could explain why and how MPS is suppressed during resistance exercise. [114]. These findings were also supported by Dreyer et al., who reported a rise in eEF2 phosphorylation due to a 75% increase in AMPK. [116]. However, there are some controversial data. For instance, Miranda et al. showed that once rat skeletal muscles were subjected to the electrically stimulated contraction, a 10-fold increase in the eEF2 phosphorylation occurred in the first minutes of contraction, while only a 3-fold growth in AMPK phosphorylation appeared 30 minutes after the onset of contraction. They reported that, it means there was no correlation between increased eEF2 phosphorylation and AMPK activation. However, there is also a possibility that the 3-fold phosphorylation of AMPK was enough for the 10-fold phosphorylation of eEF2. [117]. Furthermore, Rose et al. reported that an increase in Ca^{2+}/CaM is far more effective than AMPK on the eEF2K activity and the elongation pathway blockade. They showed that eEF2 was phosphorylated and inhibited through the Ca^{2+}/CaM -eEF2K pathway, while AMPK signaling was not involved. In addition, they stated that partial (30–40%) inhibition of eEF2K removed the curb on the protein synthesis during resistance training. They further demonstrated that during contractions, the rise in the eEF2 phosphorylation was lower in the slow-twitch soleus than the fast-twitch EDL of the mouse muscles [90]. These results are consistent with the findings that showed eEF2 levels were higher in type II than in type I fibers [118].(see table 2.)

5.2. Post-resistance exercise

It is widely accepted that MPS increases in the post-exercise period [137-139] and continues [116] up to 48 hours after exercise [140]. Several studies have shown decreased eEF2 phosphorylation during post-exercise recovery [116, 118-121, 127, 128, 141]. For example, Dreyer et al. showed that the rate of increase in MPS and eEF2 phosphorylation in the post-

exercise period (1 and 2 hours post-exercise), in young healthy individuals was independent of gender. [120]. Other researchers have shown that the increased phosphorylation of mTOR and S6K1 in the post-exercise period can overcome the inhibitory effects of AMPK on eEF2 [116]. In addition, Drummond et al. demonstrated that rapamycin administration before resistance exercise completely blocked eEF2 (Thr56) phosphorylation during post-exercise recovery, which indicated the influential role of mTORC1 in MSP [142]. Interestingly, several studies have shown that eEF2 is both rapamycin-sensitive and insensitive [34, 143]. West et al. showed that regardless of the mTORC1-dependent pathway, mTORC1-independent pathways could reduce the eEF2 phosphorylation in the post-exercise period, which was associated with myostatin down-regulation following resistance training. This presumably contributed to a prolonged rise in protein synthesis [121].

In addition, studies have reported diverse activity levels of the eEF2 signaling pathway in response to, unlike training loads. Lysenko et al. investigated the effects of moderate and high-load resistance training on the response of skeletal muscle signaling in individuals subjected to strength training. The activity of mTORC1 and its downstream factors, such as p70S6k and 4E-BP1, was increased in high-load resistance training than in moderate-load resistance training, whereas post-exercise eEF2 phosphorylation decreased after high-load exercise compared to moderate-load exercise. The increased activity of ERK1/2 might lead to a drop in eEF2 phosphorylation after high-load exercise [122]. In contrast, Burd et al. did not observe an increase in ERK1/2 and eEF2 activity following high-load resistance training in healthy recreationally active men and stated that low-load resistance exercise is a more effective factor to increase eEF2 activity compared to high-load resistance training [123]. In addition, Hulmi et al. (2010) showed that moderate-load resistance training brought about higher ERK1/2 activity than high-load resistance training. The discrepancy in ERK1/2 phosphorylation responses might be due to participants' training status [122].

On the other hand, studies carried out on resistance exercise training combined with blood flow restriction reported an increase in ribosomal S6 kinase 1 (S6K1) phosphorylation as well as a simultaneous decrease in eEF2 phosphorylation, along with a 46% rise in MSP following this type of exercise [124]. A similar reduction in phosphorylation (55%) was reported in type I [118] and type II fibers [118, 125] following resistance exercise.

5.3. Post-concurrent exercise (endurance training followed by resistance training)

Studies have also shown that aerobic exercise following resistance training results in changes in elongation pathway responses [126-128]. Pugh et al. showed that HIIT did not alter the eEF2 responses in untrained skeletal muscle immediately after resistance training [126]. Consistent with these results, Apro et al. showed a 70% reduction in eEF2 phosphorylation in the post-exercise period in healthy, moderately trained male subjects. Importantly, endurance training (30 min of cycling) followed by resistance training did not change the eEF2 phosphorylation rates compared to resistance training without prior endurance training [127]. They also examined the response of the mTORC1 and eEF2 signaling pathways following concurrent training (endurance training followed by resistance training) compared to resistance training and found an approximately 95% increase in eEF2 phosphorylation immediately following endurance training. The EF2 phosphorylation was also augmented by approximately ~55% during the post-exercise recovery period in both groups [128].

Collectively, it seems that due to the high activity of muscle fibers (especially type II fibers) during resistance training and the significant decrease in cellular energy reserve, increases in AMPK and Ca²⁺/CaM are two important factors that restrain the elongation pathway. However, the increase in Ca²⁺ and subsequent increase in Ca²⁺/CaM appear to be a more important factor. Similarly, the data also suggest that mTORC1 and ERK1/2 are two key factors

affecting eEF2 phosphorylation after post-resistance training. However, mTORC1 appears to be a more important factor, but several unknowns remain and more studies should be designed to elucidate the role of ERK1/2 in reducing eEF2 phosphorylation after resistance training.

6. Endurance exercise and control of eEF2K and eEF2

Endurance training is another well-known training method that affects MPS. Several studies have shown that endurance training solely increases mitochondrial protein synthesis and has no effect on myofibrillar protein synthesis, which leads to muscle hypertrophy [144-147]. Nevertheless, a rise in myofibrillar protein synthesis has been shown [148]. It seems that the intensity and duration of endurance exercise act as the key components that affect the MPS rate. These two factors are responsible for producing different responses in both elongation and initiation steps. The effects of these two critical factors were discussed in the following sections.

Table 3. The Summary of research investigating eEF2 and eEF2K responses to endurance exercise in skeletal muscle. *

Study	Type of exercise	Results
Rose et al [89]	Endurance: 90 min of continuous aerobic	1) skeletal muscle eEF2k was not altered by exercise 2) 5- to 7-fold increase in eEF2 phosphorylation during continuous exercise

		3) There is no correlation between increased eEF2 phosphorylation and increased AMPK activity.
Mascher et al [149]	Endurance: ergometer cycling exercise for 1 h	1) eEF2 phosphorylation decreased by 35–75% in post-exercise recovery period 2) Increasing eEF2 activity after endurance training is probably done through the mTOR or ERK1/2 pathway.
Atherton et al [141]	Endurance & Resistance: electrically stimulated with high or low frequency	1) eEF2 activity significantly increased following resistance exercise 2) eEF2 phosphorylation increased following endurance exercise
Rose et al [150]	Endurance: bicycling exercise for 30 min & bicycling exercise for 120-s	1) eEF2 phosphorylation increased during endurance exercise 2) eEF2 phosphorylation increases regardless of intensity and time of exercise. 3) There is no correlation between increased eEF2 phosphorylation and increased AMPK activity. 4) after high-intensity exercise eEF2 phosphorylation increased only in type I fibers 5) eEF2 phosphorylation decreased in the post-exercise period

*(in all studies eEF2K phosphorylates eEF2 at Thr56).

6.1. During endurance exercise

Studies show that eEF2 phosphorylation increases during endurance exercise. Rose and colleagues [89] showed that eEF2 phosphorylation increased by 5 to 7-fold during endurance

exercise (90 minutes of submaximal cycling), but eEF2k activity did not change. It was suggested that the increase in eEF2 phosphorylation was due to the inhibition of eEF2k through the Ca^{2+} -calmodulin-eEF2 kinase pathway [89]. Another study that was done on the same group [150] examined the rate of eEF2 phosphorylation at different intensities of endurance exercise, duration of exercise, and muscle fiber type and reported that during endurance exercise, eEF2 phosphorylation increased regardless of intensity and duration of exercise. They stated that, as in the previous study [89], there was no meaningful correlation between increased eEF2 phosphorylation and increased AMPK activity. They chose a high-intensity exercise activity (a 120-s bicycle test at 110% of peak work rate). To evaluate the amount of eEF2 phosphorylation in different muscle fibers, high-intensity exercise activates both muscle fiber types [151, 152]. After the protocol was done, they demonstrated that eEF2 phosphorylation just increased in type I fibers, whereas eEF2 phosphorylation was slightly reduced in type II fibers. In addition, they showed that, in the resting state, the eEF2 phosphorylation rate in type II fibers was nearly 55% more than in type I fibers [150].

6.2. Post-endurance exercise

There are conflicting results about the elongation pathway changes after endurance training. Ahtiainen et al. (2015) reported interesting data about eEF2 activity when they investigated the type (endurance or resistance) and volume (high or low) of exercise on protein synthesis signaling pathways. They demonstrated that eEF2 phosphorylation decreased in male volunteers following high-volume resistance exercise (10×10 RE), while no changes were shown following low-volume resistance exercise (5×10 RE) and endurance exercise [119]. Contrary to these results, Mascher et al. (2007) observed that eEF2 phosphorylation decreased by 35–75% in the post-endurance exercise recovery period in human muscle. They showed that 30 minutes after the activity, eEF2 phosphorylation began to decrease, then reached a peak within one hour. It was suggested that increasing eEF2 activity after endurance training

probably occurred through the mTOR or ERK1/2 pathway [149]. In another study, Rose and colleagues [150] showed that, following incremental and constant load endurance exercise, eEF2 phosphorylation starts to reduce 30 min post-exercise [150]. Furthermore, Atherton et al. observed an increase in eEF2 phosphorylation in isolated rat muscles after endurance exercise (up to 3 h after). However, they showed that after resistance training (directly and 3 h after), eEF2 phosphorylation was significantly reduced [141].

Collectively, the data show contradictory results concerning endurance training. However, it seems that like resistance training, eEF2 phosphorylation increases during endurance training following more activity of the Ca²⁺/CaM-eEF2K pathway. Further, current evidence suggests that the quantity of reduction in eEF2 phosphorylation following endurance exercise was not the same as in the post-resistance training period. Nevertheless, the inconsistent data highlights the need for further investigation to shed light on the precise mechanisms involved in this pathway.

7. Conclusion

In brief, our study has suggested that the translation elongation pathway and MPS become inactive during resistance and endurance exercise, most probably through the Ca²⁺/CaM-eEF2K pathway. They then become active in the post-resistance exercise period, possibly via the mTOR or ERK1/2 pathway. In this regard, it is not easy to make clear conclusions since nutritional status and nutrient×exercise interactions are other metabolic processes that can thoroughly influence MPS and the translation elongation pathway regardless of training status. To sum up, we inferred that these factors should be considered for a comprehensive understanding of this pathway and its underlying mechanisms.

Competing of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Study design: K.S, K.R. writing the first draft K.S, writing & database searching: K.S, M.A, editing and English proofreading K.S, K.R, G.H, M.S.P, All authors proofread and confirm the last version of the manuscript.

As this work is a collaborative effort, all authors contributed to the majority of the work. All authors have proofread and approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring questions related to the accuracy or integrity of any part of the work. All persons designated as authors qualify for authorship, and all those qualified for authorship are listed.

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*(It is highly advisable that do not use the words "article or paper" before acceptance. Usually "manuscript" would be the better choice if needed.

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