

## Research Article

# Exercise and Nano-curcumin supplementation mediates cross-talk between MAPK/ERK signaling in the regulation of inflammatory disease

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## Abstract

Impaired cell internal settings and excessive proliferation causes the occurrence of diverse ranges of syndrom and diseases. The pathological stress underlying these conditions triggers persistent flux through multiple intracellular signaling pathways amongst MAPK/ERK as master regulator. Regarding the anti-inflammatory effects of muscle contraction induced myokines and nano-curcumin supplementation, we aimed to investigate the effects of aerobic training and nano-curcumin supplementation on RAS and ERK gene expression in rat muscle tissue. In this experimental study, 32 male wistar rats (aged 4-6 weeks, 130-150 g) were randomly assigned into 4 groups, including Control (C), Moderate Intensity Continuous Training (MICT), Nano-Curcumin Supplementation (NCS) and Moderate Intensity Continuous Training + Nano-curcumin (MICT+NCS). The training groups implemented the MICT protocol consisted of running at a velocity of 18-20 m/min, 5 days a week and for a total time of 4 weeks. The Supplement groups received 80 mg/kg/day through oral gavage. Regarding the results of one-way ANOVA, 4 weeks of moderate intensity aerobic exercise and Nano-curcumin supplementation led to a significant difference in the RAS ( $P=0.001$ ) and ERK ( $P=0.01$ ) gene expression levels in muscle tissue of rats among the study groups. Also, the results of the Bonferroni test showed that implementation of 4 weeks of MICT along with nano-curcumin supplementation alleviated the RAS/ERK gene expression levels, meanwhile nano-curcumin more efficiently down-regulated the pathway; suggesting that nano-curcumin can be an effective ergogenic aid for improving anti-inflammatory properties through RAS/ERK signaling pathway.

**Key Words:** Aerobic exercise, Nano-curcumin, RAS, ERK

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## Introduction

Numerous physiological activities, including cell division, cell specialization, programmed cell death, cell survival, cell movement, metabolism, response to stress, and inflammation are linked to Mitogen-activated protein kinase (MAPK) pathways and play a role in various cellular activities (Kramer & Goodyear, 2007). Chronic exposure to inflammatory status and metabolic dysregulation of this kinase can result in the suppression of destructive reactions and the development of significant symptoms of diabetes, cardiovascular disease, and cachexia (Kramer & Goodyear, 2007). The pathogenic stress underlying these disorders causes sustained flow via many intracellular signaling channels, which coordinately enhance the sick state (Kramer & Goodyear, 2007). The most prevalent signaling cascade with the milestone of metastasis is governed by multi-kinase inhibitors is the mitogen-activated protein kinase (MAPK) pathway, encompassing the RAS–RAF–MAPK kinase (MEK)–extracellular signal-related kinase (ERK) pathway (Guo et al., 2020). The Ras/Raf/MAPK (MEK)/ERK pathway is the primary signaling cascade among all MAPK signal transduction pathways, and it has a vital function in controlling fundamental cellular processes such as cell proliferation, differentiation, stress responses, survival, and growth of tumor cells (Guo et al., 2020). ERK, also known as extracellular signal-regulated kinase 1/2, is a member of the mitogen-activated protein kinase (MAPK) family that plays a crucial role in fundamental biological processes such as cell proliferation and differentiation (Guo et al., 2020). ERK abnormalities have detrimental effects on cells and therefore, on the overall functioning of the body, owing to the significant role played by the ERK cascade (Song et al., 2023). Over-activation of proteins and kinases in the ERK pathway has been demonstrated to cause a diverse range of diseases, such as cancer, inflammation, developmental disorders, and neurological disorders (Guo et al., 2020; Song et al., 2023); Hong et al show that the levels of MAPK1 and ERK expression in ovarian cancer tissues were significantly elevated in comparison to surrounding normal tissues (Hong et al., 2018).

Polymorphisms in the RAS/ERK signaling pathway have been reported as the main contributor to cancer (Moon & Ro, 2021), neurodegenerative disease (Sun et al., 2015), and cardiovascular disease (Moens et al., 2013). Also, Statistically, 33% of RAS mutations have been observed in all human cancers thus far (Maik-Rachline et al., 2019). Therefore, studying RAS/ERK as a potential target for inflammatory treatments (Guo et al., 2020; Hong et al., 2018; Maik-Rachline et al., 2019; Moens et al., 2013; Moon & Ro, 2021; Song et al., 2023; Sun et al., 2015).

There are several methods to modulate the inflammatory status of the body; among the non-invasive approaches, we can mention the implementation of physical activity. However, the modulating effects of exercise training on immunological and oxidative stress responses are widely recognized across body fluids, organs, and tissues (Nader & Esser, 2001). Some studies have shown that physical activity increases the gene expression of ERK and RAS proteins. In most of these studies, ERK response to one or more activity sessions has been examined immediately after the activity. For instance, the in-situ studies (Nader & Esser, 2001), cycling protocols in the exercised and non-exercised people, and resistance and power exercises (Creer et al., 2005) have shown ERK is activated immediately after the activity. Nemati et al (2019) showed an increase in ERK after eight weeks of resistance exercise in the rats' flexor muscles of the big toe (Nemati et al., 2018). Xie et al (2008) also observed an increase in RAS protein levels in rats' skin tissue in reaction to a 60-minute activity session of high-intensity exercise on a running wheel (Xie et al., 2007). On the contrary, Mirdar Harijani and Mousavi (2020) reported the invariability of RAS after 12 weeks of rats' lung sub-maximal swimming exercise exposed to NNK carcinogen (Mirdar Harijani & Musavi, 2020). This discrepancy could be attributed to the intensity and duration of the exercise protocol; Several kinds of myokines are secreted from muscle fiber during contraction; so, muscle contraction has cross-talk with several key down-regulatory peptides, in a way that implementation of acute/high-intensity exercise has shown that When certain substances are introduced, they can potentially trigger the activation of MAPK, which in turn affects the transcriptional balance of redox status in skeletal muscle and leads to an increase in NF-KB levels; on the other hand, low to moderate exercise training is linked to modifications that enhance muscle oxidative capacity, such as the activation of PGC-1 $\alpha$  and fiber-type transformation, which facilitate mitochondrial biogenesis (Kramer & Goodyear, 2007).

As well, other non-invasive and non-pharmacological methods that lead to improvement and adjustment of inflammatory and related disorders are taking benefits of herbal supplementation. Curcumin is a natural phyto existing in the CURCUMA LONGA plant, a member of the ginger family, and is predominantly known

as turmeric. Given the fact that no special toxic side-effects have been reported of this natural by-product; the consumption of this product is considered in treatment regimes in inflammatory diseases as a well-known compound factor and drug supplementation (Aggarwal, 2010). Also, the application of curcumin Nanoparticles is an effective method in the treatment of cancer, causing cellular replication suppression and apoptosis imposition in a wide range of tumoral cells via unknown mechanisms, performs its anti-inflammatory and pharmaceutical activity to a large extent by inhibiting defensive signaling routes of NF-KB, PPAR-Y, CYCLIN-D1 and PI3/AKT (Mundekkad & Cho, 2023). By imposing apoptosis via releasing cytochrome C and by affecting the P53 protein, curcumin prevents metastasis progression (Mundekkad & Cho, 2023). Sandhiutami et al (2021) in their study observed that the prescription of sys platin and Nano-Curcumin led to a considerable reduction in ovarian tumors. They hence concluded that Nano-Curcumin, which is administered as a joint treatment with Sys Platin, has a treatment potential in ovarian cancer models via crosstalk with PI3K that reduces signaling routes regulation of PI3K/AKT and subsequent JAK/STAT3 (Ababd et al., 2023).

Despite all the conquered new knowledge and complex etiology of tumorigenesis signaling pathway, linking exercise training along with nano-curcumin supplementation to the modulation of inflammation symptoms, the puzzle is still incomplete and several pieces of information about signaling pathway factors in the skeletal muscle seem to be missing. We hypothesize that the adoption of nano-curcumin supplementation along with moderate-intensity continuous training may ameliorate the Ras/ERK signaling pathway through a cross-talk between muscle contraction-mediated peptides and inflammation adjustment; so we aimed to investigate the effects of MICT and nano-curcumin supplementation on gene expression levels of Ras/ERK in rat's skeletal muscle.

## Materials and Methods

### Animals

In this experimental study, 32 male Wistar rats (aged 4-6 weeks, 130-150 g) were obtained from the Razi Center of Animal Institute, Karaj Medical University. Animals were allowed food and water ad libitum before and during the experiment. Upon delivery to our laboratory animal experiment location, rats were provided 1-week interval for acclimation to the environment (24  $\pm$  1  $^{\circ}$ C; humidity of 45% to 55%, 12:12 dark/light cycle), with free access to water and standard chow (comprising of 54% mixed carbohydrate, 19% protein and 30% lipid). After this period, the rats were divided into 4 groups Control (C), Moderate Intensity Continuous Training (MICT), Nano-Curcumin Supplementation (NCS), and Moderate-intensity Continuous Training + Nano-curcumin (MICT+NCS). Animal care and ethical principles were

based on the Guide for the Care and Use of Laboratory Animals (Care & Animals, 1986) approved by the Ethics Committee of Islamic Azad University, eastern Tehran branch [IR.IAU.SRB.REC.1400.055].

## Aerobic exercise training protocol and load measurements

After a week of adaptation to treadmill running (3 times/week, 10-min/session), MICT groups performed 18 m/min for a total time of 20 min a day at 0% incline for 4 weeks, 5 sessions per week on a motorized treadmill (Iranian Model, 14 lanes, designed by Prof. Abbass Ghanbari-Niaki). Two days after the habituation period, all rats performed 30 min of training after a 15-minute warm-up, 5 days/week for 4 weeks. MICT rats were subjected to running training at 20 m/min for 30 min on a treadmill with no incline (Okamoto et al., 2021) (Table 1). To prevent the possible effect of electric shock on the research findings, during the familiarization stage, the animals were taught to avoid approaching and resting at the end part of the treadmill by conditioning to sound an alarm. The exercise training program was done in the active (dark cycle) cycle. To reduce the stress of the cages, the sedentary rats were kept out of the cages on a stationary treadmill for the same period of exercise as the training groups.

## Nano-Curcumin supplementation

The dose of nano-curcumin supplementation was chosen in accordance with the weight of the rats (80 mg/kg/day) diluted in 1.0 mL of distilled water daily for 4 weeks through oral gavage (Vijayakurup et al., 2019).

## Tissue extraction

After spending 4 weeks of treatment, the rats were anesthetized in the fasting state (12 hours) through intraperitoneal injection of a combination of ketamine and xylazine (80 and 12 mg/kg, respectively) 48 hours after the last exercise session. Soleus tissue was excised and separated via the gap on the lateral dorsal region of the lower limb and immediately froze with liquid nitrogen and stored at -80 °C until processing for subsequent real-time (RT) polymerase chain reaction (PCR) assessment (Fathi et al., 2020).

## RT-PCR analysis

Briefly, total RNA was isolated from soleus tissue by a total RNA

**Table 2.** RT-PCR forward and reverse primer sequences.

Gene	Reverse and forward primer	NCBI accession number
<b>Ras (Hras)</b>	F-5'-TGTTACCACTGGGACGACA-3' R-5'-TCTCAGCTGTGGTGAAG-3'	NM_001130442.2
<b>Ephb1 (ERK)</b>	F-5'-ATGCGCTTCACTGTGAGAGAC-3' R-5'-ATTCCGAGTAAGAGGCCCAA-3'	NM_001038663.1
<b>GAPDH</b>	F-5'-ATCAGGTTACTTTCTTGTTCAGCG-3' R-5'-TGATGCCCTGGTCAGTCTTG-3'	NM_012520.2

RNA extraction mini kit (Favorgen Biotech, Taiwan) as described in the supplier's protocol. The products were then treated with DNase I Kit (Sinaclon, Iran) to remove possible DNA. First-strand cDNA was synthesized from RNA samples with MMLV reverse transcriptase (YTA, Iran), utilizing oligo (dT) primer and dNTP mix according to the manufacturer's protocol. In this study, specific primers (RAS, ERK, and GAPDH) were designed with Primer Premier 5 software. The primer sequences used in this study have been presented in Table 2. Real-time Polymerase Chain Reaction (PCR) was performed using RealQ Plus 2x Master Mix Green (Ampliqon, Denmark) in Rotor-Gene 6000 (Corbett Research, Australia). The thermal cycling conditions were followed with 95 °C for 15 min, 40 cycles at 95°C for 30s, 60°C for 30s and 72°C for 20s. The expression level of each sample was normalized against the expression of GAPDH as a suitable internal control (Fathi et al., 2020). The relative expression level was also calculated using the comparative CT method 2- $\Delta\Delta CT$  (Livak & Schmittgen, 2001).

## Statistical analysis

All data were presented as means  $\pm$  SEM. Groups comparisons were achieved using one-way ANOVA, followed by the Bonferoni post-hoc test. Statistical significance was considered at  $P < 0.05$ . The statistical analysis was performed using SPSS statistical software (Version 22).

## Results

### Final body weight and weight changes

The results show a significant difference in the final weights of study subjects ( $P = 0.021$ ). Among all the interventions, MICT ( $P = 0.046$ ) and MICT+NCS ( $P = 0.037$ ) were able to modify the final body weight in comparison to the NC group (Table 3).

**Table 1.** MICT training protocol.

Variable	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week
<b>Intensity</b>	20 m/min	20 m/min	20 m/min	20 m/min
<b>Duration</b>	30 min/day	30 min/day	30 min/day	30 min/day

**Table 3.** Initial and final Body weight of the study rat models.

	NC (n=8)	MICT (n=8)	NCS (n=8)	MICT+NCS (n=8)	P <sub>Value</sub>
<b>Pre</b>					
weight (g)	235.7 ± 9.38	208.1 ± 13.54*	241 ± 11.87	201.7 ± 9.30*	0.021*
<b>Post</b>					
weight (g)	147.2 ± 13.06	149 ± 8.35	141 ± 10.11	139 ± 8.35	0.44

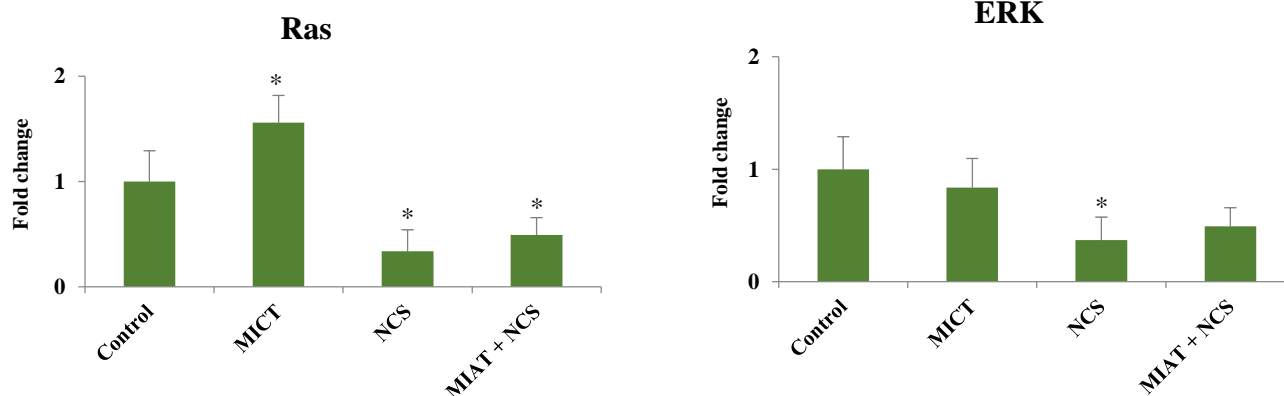
Data expressed as Mean ± SD; \* significant group difference at  $P < 0.05$  and \*\* at  $P < 0.01$  according to ANOVA. a versus the NC group ( $P < 0.05$ ).

The results of ANOVA showed that 4 weeks of Nano-curcumin supplementation and moderate-intensity aerobic exercise led to significant changes in the Ras gene expression levels ( $P=0.001$ ) among the study groups; according to the results of Bonferroni test, RAS expression in the MICT group increased after moderate-intensity exercise ( $P=0.007$ ); In addition, Nano-Curcumin supplementation has an inhibitory effect on RAS expression ( $P=0.001$ ), So the MICT+NCS group showed reduced RAS expression ( $P=0.018$ ), which may be due to the effect of Nano-Curcumin supplementation, but no exercise. As a result, the findings of MICT and Nano-curcumin supplementation are contradictory. Meanwhile, the combination of MICT and nano curcumin appears to be more similar to the impact of nano curcumin Also, Nano-curcumin supplementation showed the greatest effects on modulating RAS gene expression levels (Figure 1). Additionally, the results of ANOVA revealed a significant difference in the ERK gene expression levels in the muscle tissue of rats ( $P=0.01$ ). The results of the Bonferroni test showed that ERK variations in response to MICT ( $P=1$ ), NCS ( $P=0.018$ ), and MICT+NCS ( $P=0.87$ ) groups was decreased in comparison to the control group; However, this reduction was significant just in NCS groups compared to the control group (Figure 1).

## Discussion

The results displayed that moderate-intensity aerobic exercise by itself caused an increase in RAS and the combination of the moderate-intensity aerobic exercise with the reception of Nano-

Curcumin supplementation caused a significant reduction of the RAS gene in rats' muscle cells. The interruption in the ERK/MAPK route exists in the majority of inflammatory diseases. This route can be a proper potential target for the treatment of probable inflammation status. The RAS/RAF/MEK/ERK cascade of cell surface receptors, transfers signals into transcription factors that cause gene expression regulation, as well as regulating the activities of the proteins involved in apoptosis (Song et al., 2023). When the RAS protein is activated, it activates a protein called BRAF which is the key member and the initiator of the MAPK route (Guo et al., 2020). The kinase cascade that begins from BRAF enables MEK and kinase MAP family proteins following the activation (phosphorylation) to both activate parallel routes including PI3K to pass through the core membrane and to cause the gene expression of the cellular division via activating the transcription factors (Roberts & Der, 2007). In line with the result of the present study, Xie et al (2008) in a survey reported an increase in the RAS protein levels in rats' skin tissue in response to a 60-minute activity session of high intensity over the running wheel (Xie et al., 2007). On the contrary, Mirdar Harijani and Mousavi (2020) reported that 12 weeks of submaximal swimming exercise showed no significant variation in the rats' RAS subjected to NNK carcinogen (Mirdar Harijani & Musavi, 2020) which is not in line with the results of the present study. Reviewing the findings of various studies on cellular replication response to physical activity, the result is obtained that the variations of such indices are likely to occur in response to physical activity under a physiological situation dependent on intensity and duration. It has been shown that muscle contraction released myokines, due to cross-talk with downregulatory markers of MAPK, induces expression of peptides like P38, JNK, and PGC1 $\alpha$  in the body fluids (Kramer & Goodyear, 2007); exercise causes brief but robust perturbations in cellular stress, such as increases in ROS and metabolism that exerts catabolic responses like increasment of JNK and/or NF-KB that exacerbate inflammatory condition through induction of acute-phase proteins



**Figure 1.** Effects of Nano-Curcumin supplementation and aerobic training on Ras/ERK gene expression of soleus tissue. Data expressed as Mean ± SD; \* significant group difference at  $P < 0.05$  according to ANOVA.

and proinflammatory results from aerobic training (Kramer & Goodyear, 2007). It could be speculated that aerobic exercise has not been able to create a strong stimulus to exert antioxidant responses like RAS/ERK decline during 4 weeks of the study protocol. The results displayed that Nano-Curcumin and the combination of exercise along with Nano-Curcumin caused a significant reduction of RAS. In line with the results of the present study, Cao et al (2015) displayed that via activating the RAS/ERK signaling route and reducing the caspase cascade, curcumin prevents AGS cells from growing and it causes apoptosis and curcumin might be a potential target for the treatment of liver metaflammation (Cao et al., 2015). Gao et al (2011) concluded that Curcumin, reduced intra-cellular signaling proteins of RAS, B-RAF, P-MEK, P-ERK, C-FOS, and EGR-1 (Gao et al., 2011). By inhibiting the NF-KB route, Curcumin inhibits the anti-apoptosis gene expression and increases the Bax apoptosis protein expression (Hosseini et al., 2017). Inhibition of the WNT route, which plays a dominant role in cellular differentiation and tumor generation, curcumin reverses the process of apoptosis (Hosseini et al., 2017). Inflammation is one of the first stages associated with redox homeostasis perturbation where Curcumin causes an increase in HMOX1 and GCLM gene expression and a reduction in EGR1, PTGS2/COX2 (SYNTHASE2) and chemokines of CXCL-1 and CXCL-2 (Asgharzadeh et al., 2016). Inhibition of the route associated with  $\alpha6\beta4$  integrin, inhibiting the imposition of TGF- $\beta$ 1 and inhibiting the MMP-9 expression, Curcumin decreases the movement of cancer cells, as well as their invasive power (Tajbakhsh et al., 2018).

Another result of the present study displayed that moderate-intensity aerobic exercise along with Nano-Curcumin supplementation caused a significant reduction of the ERK gene in rats' skeletal muscles. The ERK kinase protein is phosphorylated and activated via the residuals of threonine and tyrosine. This protein activates many transcription proteins and factors with various purposes. The activation of ERK causes the expression of over 600 genes and its impact on gene transcription expression is applied either through the activation of messaging routes in the cytoplasm or via dimerization and displacement to the core and the phosphorylation of transcription factors (Casar et al., 2009).

In cytosol, ERK applies its impacts as follows: activation of P90RSK proteins, that translocate into the core and activate the SRF transcription factors. In addition, it can also activate such factors as ELK1, ATF2, AP1, CREB, and MEF2C in the cell nucleus (Ma et al., 2007). The activity of this transcription factor helps with the regeneration of muscle cells and repairing muscular damage by expressing the genes involved in numerous procedures such as mitochondrial biogenesis, vessel creation, or various types of cellular replication of mitoses, muse, and post-mitoses performance like differentiation, apoptosis and D cyclin

expression (Melo-Lima et al., 2015). MAPK route, ERK, in particular, could be activated/affected by many factors. In addition, different physiological conditions derived from physical activity, such as muscle contraction-induced growth factor secretion, RNOS production, and PH reduction are also able to conduct such activation (Wretman et al., 2001). In line with the results of the present study, Wencker et al (2012) reported that six weeks of treadmill running exercise caused a reduction of ERK in rats (Wencker et al., 2003). On the contrary, Nemati et al (2018) reported that eight weeks of resistance exercise created no significant variation in ERK in rats' flexor muscles of the big toe (Nemati et al., 2018). In addition, Thompson et al (2003) displayed that after 48 hours of eccentric activity of the biceps, ERK content maintains its significant increase trend (Thompson et al., 2003). Justifying this matter, the type of activity can be addressed. The eccentric contraction creates more muscular damage than other contractions. It has been shown, in the studies, that muscle fiber damage, causes an increase of free radicals and releasing of inflammatory factors: factors that maintain their increase after the first activity session as well (Thompson et al., 2003). Jiang et al (2015) have shown that muscular damage, activates lipin-1 protein, one of the upstream factors of the ERK messaging route. The lipin-1 protein causes the phosphorylation with ERK, forming a complex together that translocates into the cell nucleus and expresses cyclin D protein. Eventually, cyclin D causes the renovation of the damaged muscle cell (Jiang et al., 2015). The results illustrated that Nano-Curcumin and the exercise plus Nano-Curcumin compound caused a significant reduction of ERK. In line with the results of the present survey, Mortazavi et al (2020) reported that Nano-Curcumin prescription through mediating the ERK1/2 and JNK routes has the ability to prevent thrombocytopenia in mice undergoing chemotherapy (Farsani et al., 2020). Parallely, Zhang, et al (2019) reported that down-regulation of the signaling route activity of ERK/C-JUN and curcumin prevents endometrial cancer (Zhang et al., 2019). Also, Zhang et al (2016) reported that the combination of sorafenib and curcumin treatment significantly reversed apoptosis and downregulated AKT and ERK protein levels in the FTC133 cancer cell in comparison to Sorafenib alone (Zhang et al., 2016). In another study, Mousavi et al (2018) reported that curcumin could partially prevent cellular death caused by OHDA-6 and the reduction of ERK. In addition to the inhibition of various intra-cellular anti-oxidant mechanisms, the increased ROS production could cause some chronic inflammatory diseases via irreversible oxidative damage in nucleic acid, DNA, protein, and lipid membrane that creates interruption in cellular redox homeostasis such as cellular metabolism, message transfer, gene expression, cell replication and proliferation, and planned cellular death. Reactive oxygen species (ROS) are also generated in peroxisomes by the action of catalase, in the cytosol by xanthine oxidase and in membranes

by lipoxygenase and NADPH oxidase. The impact of Curcumin in the ERK activity reconstruction refers to its inhibitory impact on ROS production (Moosavi et al., 2018). Also, another mechanism of effect could be attributed to exercise-induced RNOS production that leads to the activation of post-translational factors; Rauf (2024) in a review study showed that ROS activates MAPK pathways that are implicated in apoptosis via the activity of JNK, p38-MAPK signaling proteins, and ERK. That's why curcumin supplementation had a more efficient modulatory effect than aerobic exercise intervention (Rauf et al., 2024). Also, in stress oxidative situations, different peptides and post-translational factors undergo competition for binding to the receptor; in a way that Park et al (2019) in their study showed that during Antioxidant response element (ARE) emerge, most of the antioxidant mimics like Crabp1 undergo competition with Ras to attach the receptor and trigger the downregulation signaling pathways (Park et al., 2019). It could be postulated that the different Raf/Erk alteration trends due to curcumin supplementation and aerobic exercise are due to the aforementioned mechanism described above. Finally, exercise-induced RNOS might lead to impairment in phosphorylation, acetylation, and methylation process that could be the main contributor factor in avoiding Ras activation and/or not being transferred from the cytosol to the cell nucleus and therefore its mRNA levels have not increased; In other words, the total amount of Raf in muscle cytosol probably increased, but the amount of deacetylated (and passed through the nucleus) did not increase. One of the limitations of this study is lack of western blot technique application in protein content measurement inside the muscle. The results displayed that moderate-intensity aerobic exercise by itself caused an increase in RAS and the combination of the moderate-intensity aerobic exercise with the reception of Nano-Curcumin supplementation caused a significant reduction of the RAS gene in rats' muscular cells. The interruption in the ERK/MAPK route exists in the majority of inflammatory diseases. This route can be a proper potential target for the treatment of probable inflammation status. The RAS/RAF/MEK/ERK cascade of cell surface receptors, transfers signal into transcription factors that cause gene expression regulation, as well as regulating the activities of the proteins involved in apoptosis (Song et al., 2023). When the RAS protein is activated, it activates a protein called BRAF which is the key member and the initiator of the MAPK route (Guo et al., 2020).

The kinase cascade that begins from BRAF enables MEK and kinase MAP family proteins following the activation (phosphorylation) to both activate parallel routes including PI3K to pass through the core membrane and to cause the gene expression of the cellular division via activating the transcription factors. In line with the result of the present study, Xie et al (2008) in a survey reported an increase in the RAS protein levels in rats' skin tissue in response to a 60-minute activity session of high int-

-ensity over the running wheel (Xie et al., 2007). On the contrary, Mir dar Harijani and Mousavi (2020) reported that 12 weeks of submaximal swimming exercise showed no significant variation in the rats' RAS subjected to NNK carcinogen (Mirdar Harijani & Musavi, 2020) which is not in line with the results of the present study. Reviewing the findings of various studies on cellular replication response to physical activity, the result is obtained that the variations of such indices are likely to occur in response to physical activity under a physiological situation dependent on intensity and duration. It has been shown that muscle contraction released myokines, due to cross-talk with downregulatory markers of MAPK, induces expression of peptides like P38, JNK, and PGC1 $\alpha$  in the body fluids (Kramer & Goodyear, 2007); exercise causes brief but robust perturbations in cellular stress, such as increases in ROS and metabolism that exerts catabolic responses like increase of JNK and/or NF-KB that exacerbate inflammatory conditions through induction of acute-phase proteins and proinflammatory results from aerobic training (Kramer & Goodyear, 2007). It could be speculated that aerobic exercise has not been able to create a strong stimulus to exert antioxidant responses like RAS/ERK decline during 4 weeks of the study protocol. The results displayed that Nano-Curcumin and the combination of exercise along with Nano-Curcumin caused a significant reduction of RAS. In line with the results of the present study, Cao et al (2015) displayed that via activating the RAS/ERK signaling route and reducing the caspase cascade, curcumin prevents AGS cells from growing and it causes apoptosis and curcumin might be a potential target for the treatment of liver metaflammation (Cao et al., 2015). Gao et al (2011) concluded that Curcumin, reduced intra-cellular signaling proteins of RAS, B-RAF, P-MEK, P-ERK, C-FOS, and EGR-1 (Gao et al., 2011). By inhibiting the NF-KB route, Curcumin inhibits the anti-apoptosis gene expression and increases the Bax apoptosis protein expression (Hosseini et al., 2017). Inhibition of the WNT route, which plays a dominant role in cellular differentiation and tumor generation, curcumin reverses the process of apoptosis (Hosseini et al., 2017). Inflammation is one of the first stages associated with redox homeostasis perturbation where Curcumin causes an increase in HMOX1 and GCLM gene expression and a reduction in EGR1, PTGS2/COX2 (SYNTHASE2) and chemokines of CXCL-1 and CXCL-2 (Asgharzadeh et al., 2016). Inhibition of the route associated with  $\alpha 6\beta 4$  integrin, inhibiting the imposition of TGF- $\beta 1$  and inhibiting the MMP-9 expression, Curcumin decreases the movement of cancer cells, as well as their invasive power (Tajbakhsh et al., 2018).

The advantage of this study is the simultaneous assessment of MAPK trigger peptide and endproduct gene expression levels, allowing us to make more confident statements regarding the Ras/ERK signaling fluctuations due to the use of antioxidant sup-

-plements and aerobic exercise. Among the limitations of the present study, we can point out the lack of measurement of NF-KB and/or JNK levels as the key post-translational factor that has cross-talk with MAPK in controlling inflammation, this could have increased the generalizability of the results of the study. This suggests that future research should also examine the expression of MAPK post-translational factors for a better understanding of the Cross-talk process.

## Conclusion

We can conclude that moderate-intensity aerobic exercise caused a significant increase in RAS gene expression and a significant reduction of ERK in rats' soleus muscles. In addition, the consumption of Nano-Curcumin also caused a significant reduction of RAS and ERK gene expression. It can, thus, be stated that the consumption of Nano-Curcumin supplementation leads to the reductive trend of RAS and ERK genes that might prevent the inflammation progress. However, the combination of moderate-intensity aerobic exercise, associated with Nano-Curcumin supplementation, had a more effective role in reducing RAS and ERK and probable down-regulating of inflammation relative to any exclusive intervention. On this basis, it seems that these two interventions could be used in pre-clinical studies as a complementary strategy with an effective potential for RAS and ERK suppression and inflammation progress.

## What is already known on this subject?

Most inflammatory perturbations come up with MAP/ERK dysregulation signaling as a hallmark of disease commencement. The existing knowledge encompasses several key findings as follows; It has been well understood that muscle contraction induces the secretion of diverse ranges of peptides such as myokines from skeletal muscle known as "exerkines" that cross-talk with various vital tissues in the body, like heart, liver, white and brown adipose tissue, and the nervous system, play a crucial role in inter-organ communication and act as dominant keyregulator in metabolic reactions for different purposes amongst inflammatory regulation. Additionally, previous research has demonstrated that exercise can boost liver health by influencing antioxidant systems, adjustment of liver enzymes, and facilitating metabolite excretion. Furthermore, the effects of trace elements, such as curcumin, on the modulation of inflammatory factors in various populations, including individuals with dyslipidemia, obesity, and T2M suffering from MetS, have not been well elucidated. This body of knowledge provides a foundation for understanding the complex interplay between exercise, circulatory trace-minerals, and the liver, and its implications for metabolic health and disease prevention.

## What this study adds?

The novel finding of this study provides insights into the impact of exercise-induced myokines on the modulation of liver homeostasis; the anti-inflammatory effects of aerobic exercise have been proven, but short-term ( $\leq 4$  weeks) responses of Ras/ERK gene expression levels to this training protocol could not efficiently be modulated; So, it contributes to understand that adaptation to mid (4-6 weeks) or long-term ( $\geq 6$  weeks) more efficiently modulate this signaling pathway. Furthermore, in the attribute of fat-soluble properties of curcumin, the usage of curcumin in the format of Nano-curcumin for better ingestion/absorption, could manifest potential anti-inflammatory responses probably due to increasement of bile production. Finally, the insulin-synergize role of curcumin due to its role in the regulation of enzymes in the insulin signaling cascade, might assert anabolic adaptations in the body.

### Organ Cross-Talk Tips:

- The insulin-synergize role of curcumin due to its role in the regulation of enzymes in the insulin signaling cascade, might assert anabolic adaptations in the body.

## Acknowledgements

We hereby sincerely thank and appreciate all the people who have cooperated in this research.

## Funding

This study was extracted from the Ph.D. thesis of the first author. No funding was received for this study.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** Compliance with ethical guidelines: This study followed the ethical standards and was approved by the Ethics Committee of Islamic Azad University [IR.IAU.SRB.REC.1400.055]

**Informed consent** Not applicable

## Author contributions

Conceptualization: Z.A, N.H, M.H, A.M.; Methodology: Z.A, A.M.; Software: N.H, M.H.; Validation: A.M.; Formal analysis: Z.A, N.H.; Investigation: Z.A.; Resources: N.H, M.H.; Data curation: Z.A, N.H, M.H, A.M.; Writing - original draft: Z.A.; Writing - review & editing: N.H, M.H.; Visualization: Z.A.; Supervision: A.M.; Project administration: A.M.; Funding acquisition: A.M.

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