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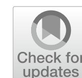

Time-course and muscle-specific gene expression of matrix metalloproteinases and inflammatory cytokines in response to acute treadmill exercise in rats

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Abstract

Background The extracellular matrix (ECM) of skeletal muscle plays a pivotal role in tissue repair and growth, and its remodeling tightly regulated by matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), and inflammatory cytokines. This study aimed to investigate changes in the mRNA expression of MMPs (*Mmp-2* and *Mmp-14*), TIMPs (*Timp-1* and *Timp-2*), and inflammatory cytokines (*Il-1β*, *Tnf-α*, and *Tgfβ1*) in the soleus (SOL) and extensor digitorum longus (EDL) muscles of rats following acute treadmill exercise. Additionally, muscle morphology was examined using hematoxylin and eosin (H&E) staining.

Methods and results Male rats were subjected to acute treadmill exercise at 25 m/min for 60 min with a %0 slope. The mRNA expression of ECM components and muscle morphology in the SOL and EDL were assessed in both sedentary and exercise groups at various time points (immediately (0) and 1, 3, 6, 12, and 24 h post-exercise). Our results revealed a muscle-specific response, with early upregulation of the mRNA expression of *Mmp-2*, *Mmp-14*, *Timp-1*, *Timp-2*, *Il-1β*, and *Tnf-α* observed in the SOL compared to the EDL. A decrease in *Tgfβ1* mRNA expression was evident in the SOL at all post-exercise time points. Conversely, *Tgfβ1* mRNA expression increased at 0 and 3 h post-exercise in the EDL. Histological analysis also revealed earlier cell infiltration in the SOL than in the EDL following acute exercise.

Conclusions Our results highlight how acute exercise modulates ECM components and muscle structure differently in the SOL and EDL muscles, leading to distinct muscle-specific responses.

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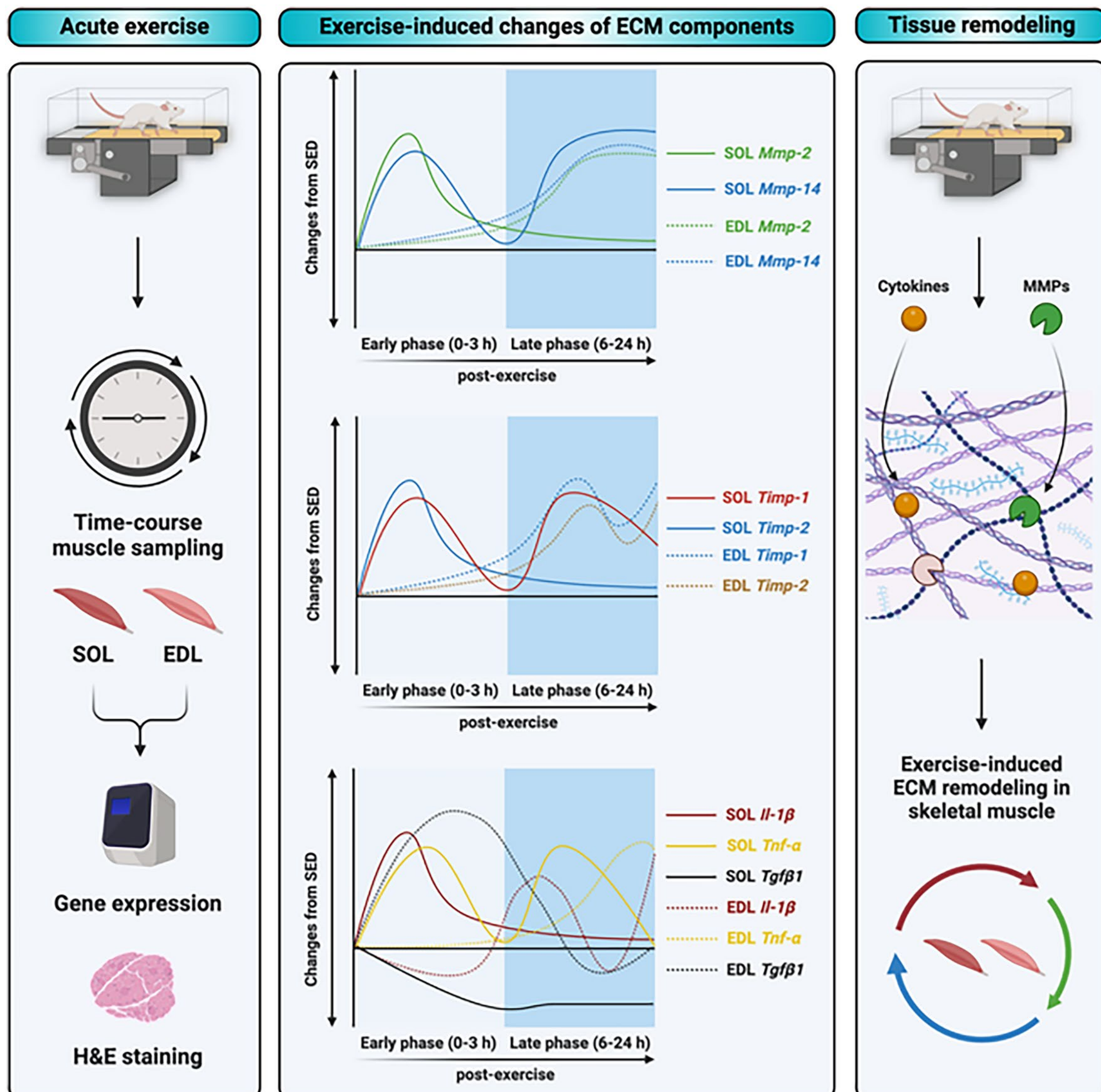
Graphical abstract

Exercise-induced skeletal muscle ECM remodeling via matrix metalloproteinases and inflammatory cytokines



Keywords Skeletal muscle · Acute exercise · Matrix metalloproteinases · Inflammatory cytokines · Gene expression

Introduction

Skeletal muscle is a highly dynamic tissue that serves to maintain posture, facilitate movement and respiration, and regulate whole-body metabolism [1, 2]. Skeletal muscle can undergo remarkable changes to meet structural, functional, and metabolic demands in response to factors, such as contractile activity, periods of disuse, nutritional status, hormonal fluctuations, and environmental changes [1, 2]. In recent years, skeletal muscle research has focused on elucidating the systemic and molecular mechanisms that control muscle contraction, as well as its physiological, biochemical, and endocrine functions [1–3]. However, muscle fibers are embedded in a dynamic and complex structure known as the extracellular matrix (ECM), which is composed of collagenous components and various macromolecules [4, 5]. Moreover, the ECM plays a pivotal role in growth, tissue repair, and the transmission of contractile forces [3, 6].

The ECM of skeletal muscle is a highly organized structure that undergoes continuous remodeling in response to injury, repair, and mechanical loading, which are mediated by specific regulatory mechanisms responsible for

ECM deposition and degradation [3]. The regulation of ECM homeostasis primarily involves two key mechanisms: matrix metalloproteinases (MMPs) and inflammatory cytokines [5, 7]. Matrix metalloproteinases are proteolytic enzymes responsible for degrading ECM macromolecules, such as collagen, elastin, and proteoglycans [8]. On the other hand, tissue inhibitors of metalloproteinases (TIMPs) are known to inhibit MMPs [6]. Notably, *Mmp-2* (also known as gelatinase A) and *Mmp-14* (also known as MT1-MMP) are well-known MMPs due to their significant involvement in skeletal muscle growth, tissue repair, and regeneration [9–11]. In addition to their role in ECM remodeling, MMPs regulate the recruitment of inflammatory cells to sites of tissue damage or injury by modulating cytokines and growth factors within the ECM [5, 7]. Moreover, MMPs produced by macrophages play a crucial role as regulators in the enzymatic cleavage of a wide range of cytokines and growth factors, including tumor necrosis factor-alpha (*Tnf-α*), interleukin-1beta (*Il-1β*), and transforming growth factor beta (*Tgfβ*) [7, 11]. Thus, understanding the interaction between MMPs, TIMPs, and inflammatory cytokines is essential to support healthy ECM remodeling under normal and pathological conditions.

The ECM of skeletal muscle is responsive to various stimuli, including contraction and exercise, leading to the upregulation of MMPs and cytokines as part of an adaptive remodeling process [10, 11]. Previous studies in rodents have shown that both mRNA and protein expression of *Mmp-2* and *Mmp-14* increase during the initial stages following electrical stimulation, thereby promoting the breakdown of ECM proteins [12, 13]. This transient elevation persists for several hours and days, indicating its significant role in ongoing remodeling processes [12, 13]. A study using mechanically overloaded rodent muscle to mimic muscle hypertrophy found that *Mmp-14* promoted the degradation of collagen, which may be crucial for ECM remodeling to facilitate hypertrophy [14]. Despite the well-known roles of MMPs and inflammatory cytokines in skeletal muscle, there is limited literature addressing the muscle-specific response to acute exercise over a 24 h period.

In this study, our aim was to investigate the effect of acute treadmill exercise on gene expression related to ECM remodeling in two distinct muscle types: SOL, predominantly composed of slow-oxidative muscle fibers (~96% type I fibers, ~3.9% type IIa fibers), and EDL, predominantly composed of fast-glycolytic muscle fibers (~5.5% type I fibers, ~18.8% type IIa fibers, ~75.7% type IIb fibers) [15]. Specifically, we sought to determine the time-course changes in mRNA expression of MMPs (*Mmp-2* and *Mmp-14*), TIMPs (*Timp-1* and *Timp-2*), and inflammatory cytokines (*Il-1β*, *Tnf-α*, and *Tgfβ1*) in both the SOL and EDL muscles.

Materials and methods

Animals

Forty-two male Sprague Dawley rats (9–12 weeks old, weighing 280–300 g) were purchased from Kobay A.S. (Ankara, Turkey). All animals were housed in groups of two per cage at a temperature of 20–24 °C on a 12 h light–dark cycle (lights on: 20:00–08:00, lights off: 08:00–20:00) with free access to a standard chow diet (Bilyem, Ankara) and water at the Animal Facility Laboratory, Faculty of Sport Sciences, Hacettepe University.

Experimental groups

After one week of acclimatization, the animals were randomly assigned to sedentary (SED, n = 6) or exercise (n = 36) group, which underwent acute treadmill exercise on a motorized rodent treadmill. Following completion of the acute treadmill exercise protocol, the animals were euthanized at six time points: immediately (0 h, n = 6), 1 h (n = 6), 3 h (n = 6), 6 h (n = 6), 12 h (n = 6), and 24 h (n = 6) post-exercise (Fig. 1). Animals in the SED group were housed under standard cage conditions.

Acute treadmill exercise protocol

Animals in the exercise groups underwent a 6 day acclimatization period on a motorized rodent treadmill with a gradual increase in running speed (from 10 to 20 m/min) and duration (from 10 to 30 min) each day. Following the acclimatization period, the animals rested for 2 days and then underwent a single session of acute treadmill exercise lasting 60 min at a running speed of 25 m/min with a 0% slope. This exercise intensity has been demonstrated to correspond to moderate-to-high intensity treadmill exercise [16, 17]. During the exercise session, the tails of the animals were stimulated with a brush to encourage them to maintain a constant speed. As rats are nocturnal animals, both treadmill acclimatization and acute exercise sessions were carried out during the dark cycle, which is the active phase for rats (at approximately between 10 a.m. and 3:00 pm) [18]. This approach aimed to minimize stress on the animals, which could otherwise affect their physiological and molecular responses [18]. Food was removed 1 h before the start of each exercise session. Following the completion of the exercise sessions, the animals were returned to their cages with free access to the food and water.

Tissue extraction

After completing the experimental procedure, the animals were euthanized under anesthesia using ketamine

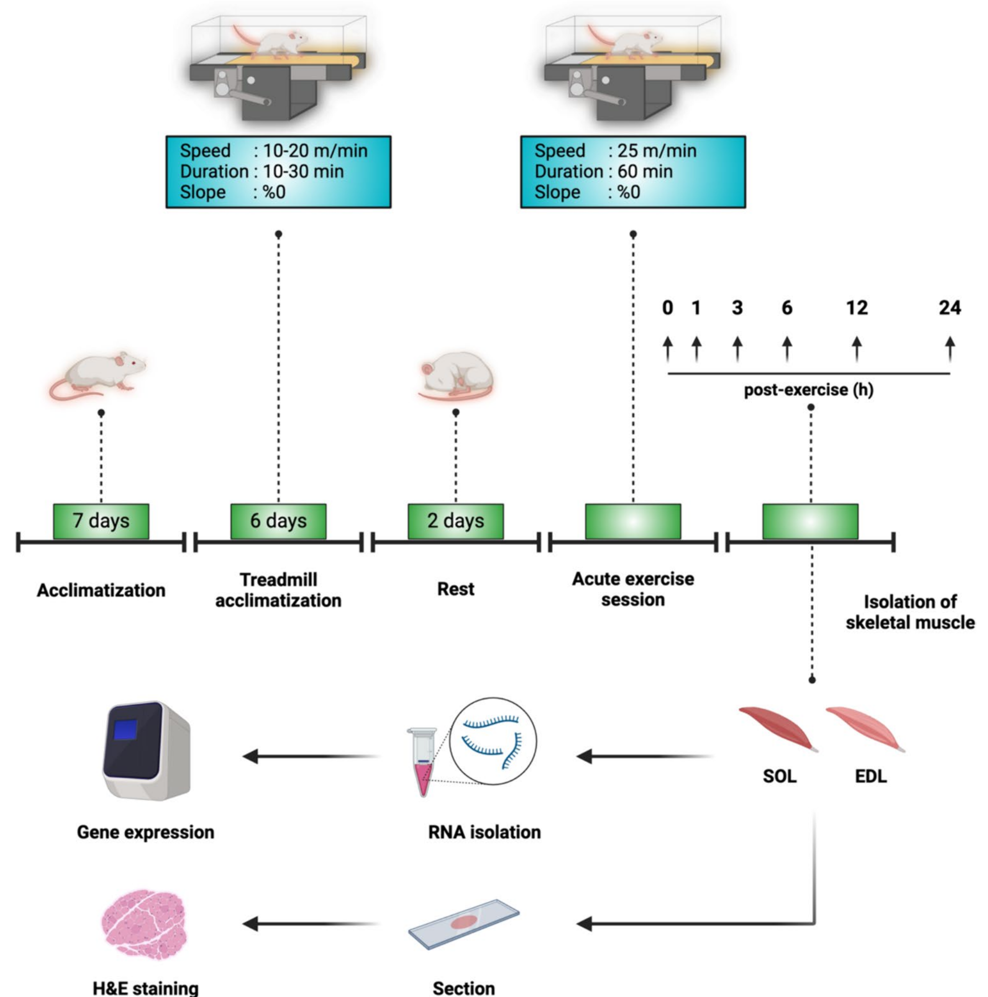


Fig. 1 Summary of the experimental procedure. The figure was created with BioRender.com

(90 mg/kg, ip) and xylazine (10 mg/kg, ip). Subsequently, the SOL and EDL were removed, immediately frozen in liquid nitrogen, and stored at − 80 °C for quantitative real-time PCR (qPCR) and histological analysis.

Total RNA isolation and cDNA synthesis

Approximately 50 mg of muscle sample was homogenized using a homogenizer (Ultraturrax T25, IKA, Germany). Total RNA extraction was performed using the Total RNA Mini Kit (catalog no: W72070, Wizbiosolutions, Seongnam, Korea) according to the manufacturer's instructions. The concentration of total RNA was quantified using a microplate reader (Thermo-Multiskan GO, USA). Subsequently, cDNA was synthesized from 100 ng of total RNA using a cDNA synthesis kit (catalog no: W2211, Wizbiosolutions, Seongnam, Korea) and cDNA was stored at − 20 °C until further use.

qPCR analysis

Gene expression analysis of target genes (*Mmp-2*, *Mmp-14*, *Timp-1*, *Timp-2*, *Il-1β*, *Tnf-α*, and *Tgfβ1*) and a housekeeping gene (*β-actin*) was performed using a PikoReal™ Real-Time PCR System (Thermo Scientific, USA) with RealQ Plus 2×Master Mix Green (catalog no: A323402, Ampliqon, Denmark). Relative gene expression levels were calculated by normalizing each gene to *β-actin* using the $2^{-\Delta\Delta CT}$ method [19]. The primer sequences used for the qPCR analysis are listed in Table 1.

Histological analysis

The skeletal muscles from each animal were fixed with 4% formaldehyde (catalog no: 722841, Adekim Kimya, Turkey) and processed using a tissue processor device (Sakura, Tissue TEK, Japan). Subsequently, 4-micron-thick sections were cut from a paraffin block using a microtome (Leica

Table 1 Primers used for qPCR

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)	Amplicon size (bp)	T_a (°C)
Il-1β	GACTTCACCATGGAACCCGT	CAGGGAGGGAAACACACGTT	200	61
Tnf-α	AGAACTCAGCGAGGACACCA	TCTGCCAGTTCCACATCTCG	200	63
Tgfβ1	GACTCTCCACCTGCAAGACC	GGACTGGCGAGCCTTAGTTT	100	55
Mmp-2	CCAGGTGCTACTTTCTCCCG	ACCAGTGCCCTCCTAAGACA	169	57
Mmp-14	GAGTATGGGAGAGTGCCACG	AAAGTGGGTATCCCCTCCGA	200	59
Timp-1	CCTAGAGACACGCTAGAGCAG	GGCACAGCTACAGGCTTTAC	110	58
Timp-2	AGAAAGTTTGCGCGGGGGAG	GTGGCCAGCAGCAGGAGG	155	57
β-actin	CTGTGTGGATTGGTGGCTCT	CAGCTCAGTAACAGTCCGCC	135	62

Il-1β interleukin-1 beta, *Tnf-α* tumor necrosis factor alpha, *Tgfβ1* transforming growth factor beta 1, *Mmp-2* matrix metalloproteinase-2, *Mmp-14* matrix metalloproteinase-14, *Timp-1* tissue inhibitor of metalloproteinases-1, *Timp-2* tissue inhibitor of metalloproteinases-2, *β-actin* beta actin, T_a annealing temperature

RM2255, Germany) and mounted on adhesive slides. These sections were then stained with H&E using a slide stainer (Sakura, Tissue TEK, Japan) and the respective solutions of hematoxylin (catalog no: 05-06004/L, Bio Optica, Italy) and eosin (catalog no: 05 10007/L, Bio Optica, Italy). The stained sections were observed under a light microscope (Nikon Eclipse 80i, Japan), and images were captured at a magnification of × 200 using NIS Element 3.0 software (Japan). Inflammatory cells infiltrating the muscles were identified in five randomly selected fields within each section.

Statistical analysis

All values were analyzed using GraphPad Prism version 8.0 (GraphPad Software, USA). One-way analysis of variance (ANOVA) with Fisher's least significant difference (LSD) post hoc test was used to assess differences among multiple groups. All values are presented as the mean ± standard error of the mean (SEM), and statistical significance was set at $p < 0.05$.

Results

Acute treadmill exercise increases the mRNA expression of MMPs and TIMPs

To investigate the impact of acute treadmill exercise on the gene expression of MMPs and TIMPs, we measured the mRNA expression levels of *Mmp-2*, *Mmp-14*, *Timp-1*, and *Timp-2* in both the SOL and EDL.

Compared to the SED, we observed an increase in *Mmp-2* mRNA expression at 0 and 1 h post-exercise in the SOL (Fig. 2A, Table 2). In the EDL, *Mmp-2* mRNA expression increased at 3 h post-exercise and remained elevated at 6, 12, and 24 h post-exercise compared to the SED (Fig. 2B, Table 2). Furthermore, mRNA expression of *Mmp-14* increased at 0, 6, and 24 h post-exercise in the SOL (Fig. 2C, Table 2), while in the EDL, mRNA expression of *Mmp-14* increased at 6 and 24 h post-exercise compared to the SED (Fig. 2D, Table 2).

The fold changes in mRNA expression of *Timp-1* and *Timp-2* are illustrated in Fig. 2 and Table 2. Compared to the SED, *Timp-1* mRNA expression increased at 0 and 6 h post-exercise in the SOL (Fig. 2E, Table 2) and at 6 and 24 h post-exercise in the EDL (Fig. 2F, Table 2). Similarly, *Timp-2* mRNA expression increased at 0 h in the SOL (Fig. 2G, Table 2) and 3, 6, and 24 h post-exercise in the EDL (Fig. 2H, Table 2).

Acute treadmill exercise differentially affects the mRNA expression of *Il-1β*, *Tnf-α*, and *Tgfβ1*

The time-course analyses of changes in mRNA expression of *Il-1β*, *Tnf-α*, and *Tgfβ1* in response to acute exercise in both the SOL and EDL are presented in Fig. 3 and Table 2. Compared to the SED, the mRNA expression of *Il-1β* increased at 0 h post-exercise in the SOL (Fig. 3A, Table 2). In the EDL, the mRNA expression of *Il-1β* decreased at 1 h, increased at 3 h, and then decreased again at 12 h post-exercise compared to the SED (Fig. 3B, Table 2).

Compared to the SED, the mRNA expression of *Tnf-α* increased at 0, 1, and 6 h post-exercise in the SOL (Fig. 3C, Table 2). In contrast, the mRNA expression of *Tnf-α* in the EDL increased at 24 h post-exercise compared to the SED (Fig. 3D, Table 2).

In the SOL, the mRNA expression of *Tgfβ1* decreased at 0, 1, 3, 6, 12, and 24 h post-exercise compared to the SED (Fig. 3E, Table 2). In contrast, the mRNA expression of *Tgfβ1* in the EDL increased at 0 and 3 h but decreased at 12 h post-exercise compared to the SED (Fig. 3F, Table 2).

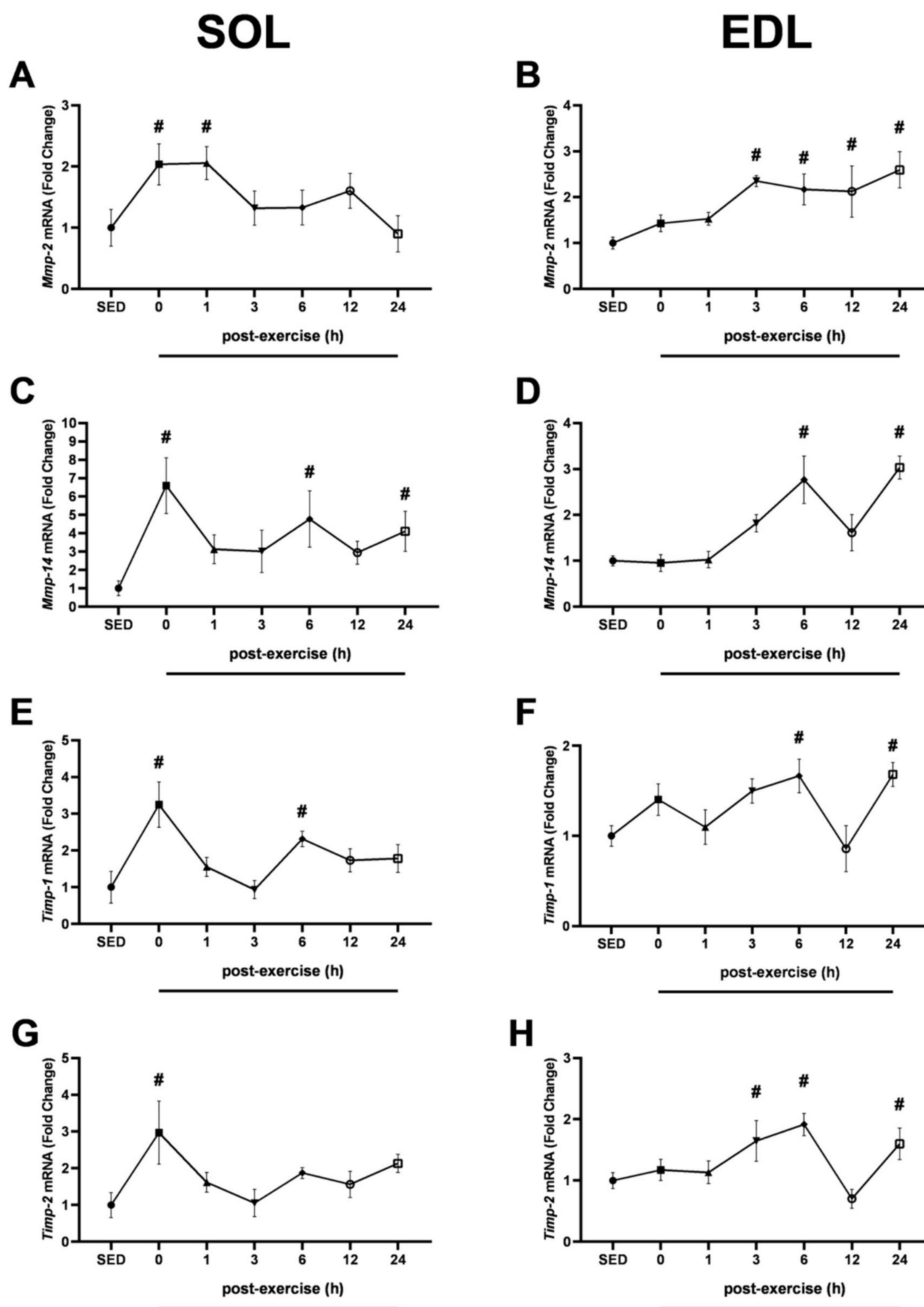


Fig. 2 mRNA expression of *Mmp-2* (**A**, **B**), *Mmp-14* (**C**, **D**), *Timp-1* (**E**, **F**), and *Timp-2* (**G**, **H**) following acute treadmill exercise at different post-exercise time points (0, 1, 3, 6, 12, and 24 h) compared to the SED in the SOL (**A**, **C**, **E**, **G**) and EDL (**B**, **D**, **F**, **H**). Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA with the LSD post hoc test. n = 6 for all time points. #Significantly different from the SED ($p < 0.05$)

Table 2 Relative fold changes in the mRNA expression of MMPs, TIMPs, and inflammatory cytokines in response to acute treadmill exercise

Gene		SED	Post-exercise (h)					
			0	1	3	6	12	24
MMPs and TIMPs								
Mmp-2	SOL	1.00 ± 0.30	**2.04 ± 0.34**	**2.06 ± 0.27**	1.32 ± 0.28	1.33 ± 0.29	1.60 ± 0.28	0.90 ± 0.29
	EDL	1.00 ± 0.13	1.43 ± 0.18	1.53 ± 0.14	**2.35 ± 0.12**	**2.17 ± 0.34**	**2.12 ± 0.56**	**2.59 ± 0.40**
Mmp-14	SOL	1.00 ± 0.40	**6.60 ± 1.52**	3.13 ± 0.79	3.01 ± 1.15	**4.77 ± 1.53**	2.94 ± 0.62	**4.11 ± 1.10**
	EDL	1.00 ± 0.11	0.95 ± 0.18	1.03 ± 0.18	1.82 ± 0.19	**2.76 ± 0.51**	1.61 ± 0.40	**3.03 ± 0.25**
Timp-1	SOL	1.00 ± 0.43	**3.25 ± 0.62**	1.55 ± 0.26	0.93 ± 0.25	**2.32 ± 0.21**	1.73 ± 0.31	1.78 ± 0.38
	EDL	1.00 ± 0.11	1.40 ± 0.17	1.10 ± 0.19	1.50 ± 0.13	**1.67 ± 0.19**	0.86 ± 0.25	**1.68 ± 0.13**
Timp-2	SOL	1.00 ± 0.34	**2.97 ± 0.86**	1.62 ± 0.27	1.05 ± 0.37	1.87 ± 0.15	1.56 ± 0.36	1.68 ± 0.14
	EDL	1.00 ± 0.13	1.17 ± 0.17	1.13 ± 0.18	**1.65 ± 0.33**	**1.92 ± 0.18**	0.70 ± 0.16	**1.60 ± 0.26**
Inflammatory cytokines								
Il-1β	SOL	1.00 ± 0.32	**5.17 ± 1.73**	2.80 ± 0.98	1.51 ± 0.44	2.11 ± 0.19	1.20 ± 0.33	2.05 ± 0.62
	EDL	1.00 ± 0.09	1.24 ± 0.08	**0.57 ± 0.17**	**1.67 ± 0.13**	1.27 ± 0.14	**0.46 ± 0.10**	1.21 ± 0.25
Tnf-α	SOL	1.00 ± 0.21	**4.35 ± 0.85**	**3.16 ± 0.90**	0.75 ± 0.25	**2.77 ± 0.33**	2.40 ± 0.63	2.05 ± 0.29
	EDL	1.00 ± 0.09	1.17 ± 0.24	0.55 ± 0.19	1.11 ± 0.07	0.81 ± 0.14	0.69 ± 0.27	**3.86 ± 1.16**
Tgfβ1	SOL	1.00 ± 0.37	**0.35 ± 0.08**	**0.10 ± 0.05**	**0.08 ± 0.03**	**0.08 ± 0.04**	**0.25 ± 0.14**	**0.07 ± 0.01**
	EDL	1.00 ± 0.12	**1.89 ± 0.15**	1.53 ± 0.49	**2.29 ± 0.09**	0.84 ± 0.31	**0.17 ± 0.06**	0.70 ± 0.30

SED sedentary, *Il-1β* interleukin-1 beta, *Tnf-α* tumor necrosis factor alpha, *Tgfβ1* transforming growth factor beta 1, *Mmp-2* matrix metalloproteinase-2, *Mmp-14* matrix metalloproteinase-14, *Timp-1* tissue inhibitor of metalloproteinases-1, *Timp-2* tissue inhibitor of metalloproteinases-2, *SOL* soleus, *EDL* extensor digitorum longus.

Values in bold indicate statistical significance compared to the SED ($p < 0.05$)

Acute treadmill exercise initiates inflammatory cell infiltration in the SOL and EDL

To assess the effects of acute exercise on muscle structure, we performed H&E staining to examine the morphology of both the SOL and EDL. Our results revealed that acute exercise did not lead to inflammatory cell infiltration into the interstitial space in the SED and immediately (0 h) post-exercise in the SOL (Fig. 4). However, in the SOL, we observed minimal inflammatory cell infiltration at 1, 3, 6, and 24 h post-exercise, with moderate inflammatory cell infiltration noted at 12 h post-exercise (Fig. 4).

In the EDL, acute exercise did not result in inflammatory cell infiltration into the interstitial space at 0, 1, 3, and 6 h post-exercise (Fig. 4). However, minimal inflammatory cell infiltration was observed at 12 and 24 h post-exercise in the EDL (Fig. 4).

Discussion

Overview of the main findings

The skeletal muscle ECM displays remarkable plasticity through remodeling of its structure and function to meet the demands imposed by muscle contraction and exercise in skeletal muscle. This plasticity of the ECM is regulated by the activation and/or repression of molecular pathways, which provide the basis for exercise adaptations. Therefore, understanding the underlying mechanisms will provide important details on the time-course and muscle-specific analysis of these molecular pathways in response to acute exercise and exercise adaptation [1–3].

To our knowledge, these are the first experiments to elucidate the time-course and muscle-specific expression of MMPs, TIMPs, and inflammatory cytokines in response to acute treadmill exercise for up to 24 h, providing important findings regarding the effects of exercise on ECM components and structure in skeletal muscle. Our results showed that acute treadmill exercise increased the mRNA levels of *Mmp-2*, *Mmp-14*, *Timp-1*, and *Timp-2* in the SOL and EDL. However, the mRNA expression of MMPs and TIMPs elevated immediately and during the early stages of the post-exercise time points in the SOL compared to the EDL. A similar trend was also observed for the mRNA expression of *Il-1β* and *Tnf-α* in the SOL compared to the EDL. In contrast, the mRNA expression of *Tgfβ1* decreased in the SOL at all post-exercise time points, while *Tgfβ1* mRNA expression initially increased at 0 and 3 h post-exercise and then decreased at 12 h post-exercise in the EDL.

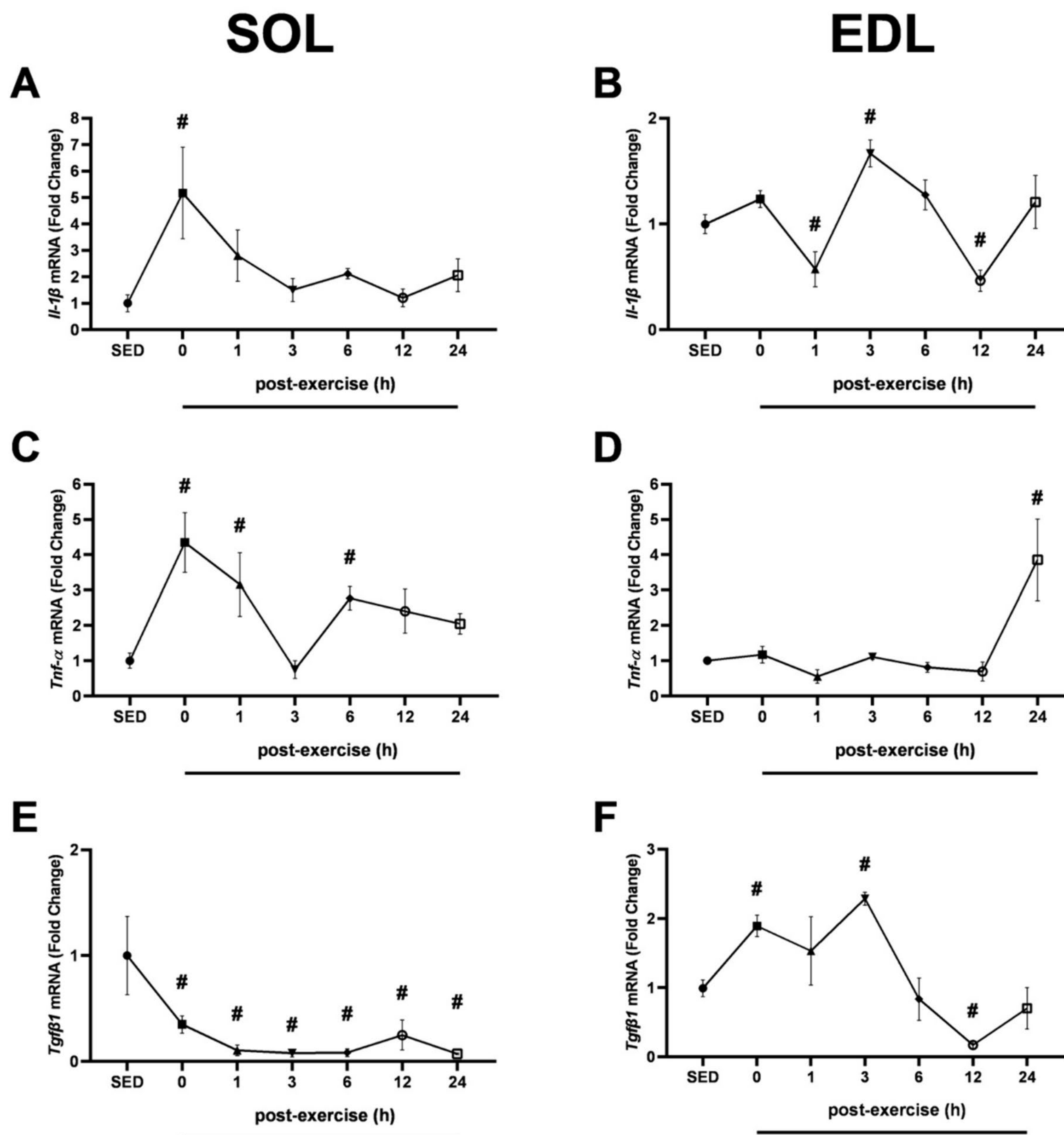


Fig. 3 mRNA expression of *Il-1β* (**A**, **B**), *Tnf-α* (**C**, **D**), and *Tgfβ1* (**E**, **F**) following acute treadmill exercise at different post-exercise time points (0, 1, 3, 6, 12, and 24 h) compared to the SED in the SOL (**A**, **C**, **E**) and EDL (**B**, **D**, **F**). Data are presented as mean ± SEM. Statistical analysis was performed using one-way ANOVA with the LSD post hoc test. n = 6 for all time points. #Significantly different from the SED ($p < 0.05$)

Acute treadmill exercise differentially induces the mRNA expression of MMPs and TIMPs in the SOL and EDL at various post-exercise time points

The maintenance of the ECM is regulated by specialized enzymes responsible for the degradation of ECM components [4, 11, 20]. Several studies have shown that *Mmp-2* and *Mmp-14* are upregulated in skeletal muscle [21–23], possibly due to ECM remodeling initiated by muscle contraction, mechanical loading, injury, and regeneration of muscle fibers [9, 24].

Our results showed that acute treadmill exercise was sufficient to increase *Mmp-2* and *Mmp-14*, as well as their inhibitors *Timp-1* and *Timp-2*, in both the SOL and EDL. These findings align with previous studies indicating that acute and short-term exercise can increase the expression of *Mmp-2*, *Mmp-14*, *Timp-1,* and *Timp-2* in skeletal muscles [23, 25, 26]. However, an early response of *Mmp-2* and

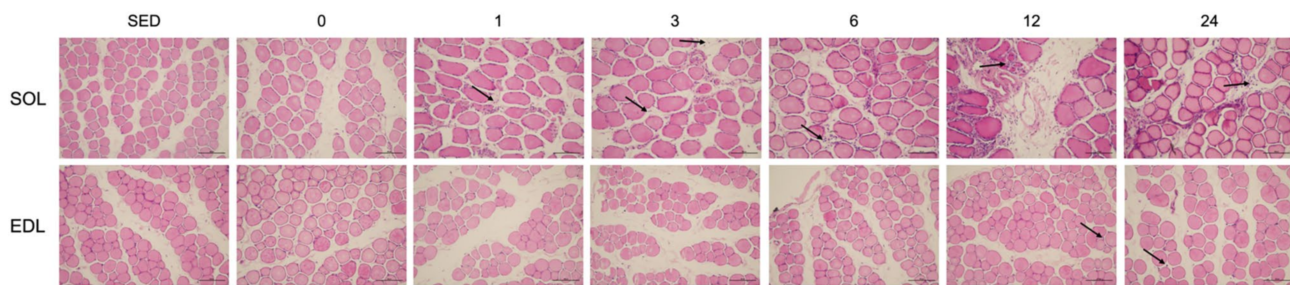


Fig. 4 Inflammatory cell infiltration in the SOL and EDL following acute treadmill exercise was evaluated at different post-exercise time points (0, 1, 3, 6, 12, and 24 h) and in the SED. Representative images of H&E staining are shown for each group at a magnification of × 200. n = 6 for all time points. Black arrows (→) indicate inflammatory cell infiltration. Scale bar = 100 μm

Mmp-14 was observed in the SOL compared to the EDL, with the induction observed at 0, 3, and 6 h post-exercise. Additionally, the mRNA expression of *Timp-1* and *Timp-2* increased at 0 and 6 h post-exercise, while a delayed mRNA response (3–24 h post-exercise) was observed in the EDL compared to the SOL, suggesting a trend similar to that of *Mmp-2* and *Mmp-14*.

The transient upregulation of MMPs is typically considered the first step in proper repair following muscle contraction, as it promotes the stimulation of regenerative capacity and activation of satellite cells [10]. Our data support previous findings regarding the induction of MMPs and provide important details on the time-course and muscle-specific response to exercise [10, 23]. One of the hallmarks of adaptation to endurance exercise is that type I muscle fibers are recruited more than type II muscle fibers during an endurance exercise session, making them more susceptible to skeletal muscle damage [2, 27]. Thus, the initiation of ECM remodeling may occur earlier in the SOL (predominantly composed of type I fibers) than in the EDL (predominantly composed of type II fibers). These effects may also be somewhat specific to collagen abundance, as slow-oxidative muscle fibers have significantly higher concentrations of collagen than fast-glycolytic muscle fibers [28, 29]. Takala et al. emphasized the significance of the fiber type-specific response of ECM remodeling to endurance training [30]. The authors also noted that collagen turnover in the quadriceps muscle of mice is more pronounced during the initial phase of exercise training in red muscles compared to white muscles [30]. This observation may explain the early-phase induction of MMPs observed in the SOL compared to the EDL, possibly due to the muscle-specific response to endurance exercise. Another possible explanation is that muscle-specific differences may also contribute to more pronounced exercise-induced ECM remodeling through the induction of MMPs and their inhibitors, TIMPs, in the SOL compared to the EDL. Immunohistochemical staining of *Mmp-2* revealed that basal intracellular levels of *Mmp-2* are much more prominent in type II muscle fibers in the gastrocnemius muscle of mice [31], suggesting that the SOL could be more sensitive to exercise-induced induction *Mmp-2 expression* than the EDL due to lower basal *Mmp-2*.

Similar to *Mmp-2*, *Mmp-14* mRNA expression has also been reported in skeletal muscle [9]; however, there is no information on whether its expression differs in type I or type II muscle fibers. In our study, the exercise-induced increase in *Mmp-14* mRNA expression was more pronounced in the SOL compared to the EDL. An explanation for the increase in *Mmp-14* could be that *Mmp-14* is required for proteolytic cleavage of *proMmp2* [6]. A single bout of exercise did not increase the mRNA expression of *Mmp-2* or *Mmp-14* in the vastus lateralis muscle, which is predominantly composed of type II muscle fibers, immediately after and 120 min following exercise [26]. However, the mRNA expression of *Mmp-2* and *Mmp-14* increased after 10 days of exercise [25]. Part of this discrepancy in the mRNA expression of *Mmp-2* and *Mmp-14* related to acute exercise might be due to the skeletal muscle fiber composition since the vastus lateralis muscle is composed of type II fibers [32]. We observed delayed mRNA expression of *Mmp-2* and *Mmp-14* in response to acute treadmill exercise in the EDL, suggesting that muscle-specific mRNA expression of *Mmp-2* was dependent on the timing of muscle isolation. Thus, further studies are needed to fully elucidate the exercise-induced changes in the mRNA expression of MMPs at multiple post-exercise time points.

Time-course and muscle-specific changes in the mRNA expression of *Il-1β*, *Tnf-α*, and *Tgfβ1* in response to acute treadmill exercise

The inflammatory response is a complex and vital mechanism that enables the elimination of pathogens and the maintenance of tissue homeostasis. Adaptive inflammation, in which stress conditions are resolved, involves the activation of immune cells that infiltrate the injury site to remove cellular debris and promote tissue repair, ultimately restoring tissue homeostasis. However, if stress cannot be resolved and

persists, maladaptive inflammation contributes to the sustained infiltration of immune cells orchestrated by cytokines and chemokines, resulting in impaired tissue function [33]. Thus, proper functioning of the inflammatory response is necessary to maintain tissue homeostasis under both physiological and pathophysiological conditions.

Acute exercise may represent a major challenge to whole-body homeostasis, caused by the contractile activity of skeletal muscles, resulting in the activation of an inflammatory response through cytokines and chemokines [1]. Here, we demonstrated that the early response of *Il-1β* and *Tnf-α* mRNA expression to acute treadmill exercise in the SOL compared to the EDL appears to be muscle-specific. Both *Il-1β* and *Tnf-α* are classic proinflammatory cytokines that are released in response to cellular damage [33]. This transient increase in the muscle-specific inflammatory response leads to the activation of several signaling pathways that contribute to the myogenesis, repair, and remodeling of the skeletal muscle [34]. Despite their important role in skeletal muscle, little is known about exercise-induced changes in inflammatory cytokine expression. A single bout of acute eccentric exercise, known to cause structural damage to skeletal muscle fibers, is a potent stimulus for the increase in *Il-1β* and *Tnf-α* in skeletal muscle [35, 36]. Importantly, muscle- and fiber-specific mRNA and protein expression of *Il-1β* and *Tnf-α* differ, as basal *Il-1β* does not change between the SOL and EDL in rats, whereas type II fibers express more *Tnf-α* than type I fibers in both rats and humans [37, 38]. These results make it difficult to provide a simple explanation for muscle type-specific mRNA expression of *Il-1β* and *Tnf-α* in response to acute treadmill exercise. However, a possible explanation is that endurance exercise causes more damage to type I fibers and results in greater recruitment of type I fibers [2, 27]. Additionally, the recruitment of type I fibers depends on the exercise intensity [2]. Consequently, different inflammatory responses can be observed owing to muscle damage and the recruitment of fiber types associated with the type and intensity of exercise.

A critical feature of *Il-1β* and *Tnf-α* is that they are highly interconnected and can influence each other at the transcriptional and protein levels. The gene expression of *Il-1β* and *Tnf-α* is bidirectional, with *Tnf-α* being upregulated by *Il-1*, while *Il-1β* stimulates the expression of *Tnf-α* via nuclear factor kappa B, a central switch of the inflammatory response [39]. Our time-course and muscle-specific analyses revealed that acute treadmill exercise resulted in an increase in the mRNA expression of *Il-1β* and *Tnf-α* in the SOL compared to the EDL in the early stage of post-exercise. This observation also suggests that the early response of these cytokines to acute exercise might exert their effects in a paracrine and endocrine-dependent manner on the target tissues, thereby promoting the delayed expression of *Il-1β* and *Tnf-α* in the EDL. Additionally, the exercise-induced inflammatory response via macrophages that express *Il-1β* and *Tnf-α* is associated with early stage regeneration to clear damaged fibers [40]. The underlying mechanism is not entirely clear, but we speculate that the increased gene expression of *Il-1β* and *Tnf-α* may trigger tissue regeneration. However, further studies are needed to elucidate the cellular and molecular mechanisms underlying exercise-induced inflammation.

In contrast to the early phase increase in the expression of *Il-1β* and *Tnf-α*, a decrease in *Tgfβ1* expression was observed at all post-exercise time points in the SOL. However, the mRNA expression of *Tgfβ1* dramatically increased at 0 and 3 h post-exercise in the EDL. These results are in line with previous findings that acute exercise increases the expression of *Tgfβ1*. For example, Heinemeier et al. reported an increase in muscle *Tgfβ1* after 1 h of one-leg kicking exercise [41]. Similar results were also reported for the gene expression of *Tgfβ1* in the gastrocnemius muscle of rats immediately after acute exercise [42] and in the EDL muscle of rats after 30 day of exercise [43]. Based on previous findings, *Tgfβ1* expression can be induced by contractile activity. The literature lacks comprehensive data on the mRNA and protein expression of *Tgfβ1* in response to exercise, especially concerning muscle-specific variations. *Tgfβ1* signaling has been identified as a key regulator of skeletal muscle regeneration, ECM remodeling, and fibrosis [44]. In the acute phase of skeletal muscle damage caused by injury or exercise, *Tgfβ1* has the potential to stimulate the production of ECM components, such as collagen and fibronectin [44, 45]. Furthermore, there is a bidirectional relationship between *Tgfβ* and MMPs, suggesting that MMPs proteolytically activate *Tgfβ,* while *Tgfβ* suppresses MMP upregulation [46, 47]. Our results are similar to those of previous studies, and it can be speculated that reduced *Tgfβ1* expression is essential for facilitating post-exercise skeletal muscle remodeling by inducing MMPs in the SOL.

Conclusion

Exercise is a powerful tool not only for maintaining the healthy state of individuals and acting as a poly-pill for disease prevention but also for improving sports performance. While the benefits of exercise are partly due to transient changes in the mRNA expression of genes within the first few hours to 24 h, adaptation to exercise occurs through changes in protein levels during prolonged, repeated acute exercise sessions. Our results suggest that acute treadmill exercise induces muscle-specific responses, with early increases in the mRNA expression of MMPs, TIMPs, *Il-1β*, and *Tnf-α* in the SOL and late upregulation of the mRNA expression of MMPs and TIMPs, as well as increased expression of *Tgfβ1* in the EDL. These results contribute to our understanding of the role of MMPs, TIMPs, and

cytokines in skeletal muscle remodeling in response to acute exercise. Given the molecular adaptations that occur in skeletal muscle in response to exercise, it is important to understand the responses that are fiber type specific.

Limitations of this study and future directions

While these findings provide important insights into exercise-induced changes in different skeletal muscle types, this study was designed to examine the mRNA expression of a limited number of ECM components in both the SOL and EDL. Therefore, it is essential to investigate the roles of proinflammatory cytokines (e.g., Il-6) and anti-inflammatory cytokines (e.g., Il-10) in both skeletal muscle and serum/plasma samples.

Given the complexity of the response to exercise, the incorporation of OMICS-based methods, which are crucial for identifying dynamic molecular networks, could facilitate a thorough characterization of gene and protein expression within skeletal muscle. The complex nature of skeletal muscle makes it difficult to analyze the response to acute exercise because of the mixed muscle fiber composition and resident cells, including satellite cells, fibroblasts, and immune cells. Therefore, single-cell analysis provides a clearer picture of the molecular changes during skeletal muscle remodeling induced by acute exercise compared to mixed muscle analysis. Finally, further research should aim to elucidate the underlying mechanisms involved in the response to exercise training and provide valuable information for long-term skeletal muscle remodeling.

Translational perspective

The skeletal muscle ECM plays a unique role in diverse biological functions, including mechanical transmission, muscle repair, and regeneration. Skeletal muscle plasticity relies on the response of ECM components to physiological and pathophysiological stressors. Moreover, maintaining skeletal muscle integrity without properly functioning ECM components and the ECM itself is challenging. There are physiological, biochemical, and anatomical differences between animal models (e.g., zebrafish and rodents) and humans, making it difficult to generalize findings from animals to humans. However, several aspects of human translation must be considered.

1. The underlying mechanism of ECM remodeling in response to exercise in the SOL and EDL in rodents may better reflect the muscle-specific response due to the difficulty in obtaining biopsies from the SOL and EDL in humans.
2. Differences in the fiber type distribution and function of each muscle fiber have been observed between the skeletal muscles of mammalian species, such as rats and humans. This difference may limit the comparison and translation of the ECM response to exercise across mammalian species [15, 48].
3. Recent studies have shown that the accumulation of ECM (e.g., increased collagen content) during obesity and aging may lead to fibrosis and the impairment of skeletal muscle function [22, 49]. Thus, the adaptive induction of ECM components may play a central role in skeletal muscle function.
4. In addition to its functions, the ECM serves as an environment that transmits mechanical force, allowing the transmission of growth signals to muscle fibers and satellite cells [20]. This signal transduction results in skeletal muscle hypertrophy, directly preventing the loss of skeletal muscle mass during aging [20].
5. Therapeutics targeting ECM components such as MMPs may have great potential and should be considered as exercise mimetics, offering significant health benefits to individuals with metabolic disorders and those with limited physical activity.

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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