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SYSTEMATIC REVIEW

Pax7$^+$ Satellite Cells in Human Skeletal Muscle After Exercise: A Systematic Review and Meta-analysis

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Abstract

Background Skeletal muscle has extraordinary regenerative capabilities against challenge, mainly owing to its resident muscle stem cells, commonly identified by Pax7$^+$, which expediently donate nuclei to the regenerating multinucleated myofibers. This local reserve of stem cells in damaged muscle tissues is replenished by undifferentiated bone marrow stem cells (CD34$^+$) permeating into the surrounding vascular system.

Objective The purpose of the study was to provide a quantitative estimate for the changes in Pax7$^+$ muscle stem cells (satellite cells) in humans following an acute bout of exercise until 96 h, in temporal relation to circulating CD34$^+$ bone marrow stem cells. A subgroup analysis of age was also performed.

Methods Four databases (Web of Science, PubMed, Scopus, and BASE) were used for the literature search until February 2022. Pax7$^+$ cells in human skeletal muscle were the primary outcome. Circulating CD34$^+$ cells were the secondary outcome. The standardized mean difference (SMD) was calculated using a random-effects meta-analysis. Subgroup analyses were conducted to examine the influence of age, training status, type of exercise, and follow-up time after exercise.

Results The final search identified 20 studies for Pax7$^+$ cells comprising a total of 370 participants between the average age of 21 and 74 years and 26 studies for circulating CD34$^+$ bone marrow stem cells comprising 494 participants between the average age of 21 and 67 years. Only one study assessed Pax7$^+$ cells immediately after aerobic exercise and showed a 32% reduction in exercising muscle followed by a fast repletion to pre-exercise level within 3 h. A large effect on increasing Pax7$^+$ cell content in skeletal muscles was observed 24 h after resistance exercise (SMD = 0.89, $p < 0.001$). Pax7$^+$ cells increased to ~ 50% above pre-exercise level 24–72 h after resistance exercise. For a subgroup analysis of age, a large effect (SMD = 0.81, $p < 0.001$) was observed on increasing Pax7$^+$ cells in exercised muscle among adults aged > 50 years, whereas adults at younger age presented a medium effect (SMD = 0.64, $p < 0.001$). Both resistance exercise and aerobic exercise showed a medium overall effect in increasing circulating CD34$^+$ cells (SMD = 0.53, $p < 0.001$), which declined quickly to the pre-exercise baseline level after exercise within 6 h.

Conclusions An immediate depletion of Pax7$^+$ cells in exercising skeletal muscle concurrent with a transient release of CD34$^+$ cells suggest a replenishment of the local stem cell reserve from bone marrow. A protracted Pax7$^+$ cell expansion in the muscle can be observed during 24–72 h after resistance exercise. This result provides a scientific basis for exercise recommendations on weekly cycles allowing for adequate recovery time. Exercise-induced Pax7$^+$ cell expansion in muscle remains significant at higher age, despite a lower stem cell reserve after age 50 years. More studies are required to confirm whether Pax7$^+$ cell increment can occur after aerobic exercise.

Clinical Trial Registration Registered at the International Prospective Register of Systematic Reviews (PROSPERO) [identification code CRD42021265457].

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Key Points

Pax7$^+$ satellite cells donate nuclei to regenerating myofibers in response to acute damage.

Pax7$^+$ cells in human muscle transiently depleted during aerobic exercise followed by a quick replenishment within 3 h.

Pax7$^+$ cell number in human muscle increases (~50%) during 24–72 h after resistance exercise.

Pax7$^+$ cell expansion occurs after a transient increase in circulating CD34$^+$ bone marrow stem cells following exercise.

Exercise-induced Pax7$^+$ cell expansion in muscle remains normal after age 50 years.

1 Introduction

Satellite cells are myogenic stem cells located surrounding myofibers between the sarcolemma and basal lamina [1–4], which contribute to muscle growth and repair by a quick fusion of their nuclei into the cytoplasm of myofibers [1, 2, 5]. Lower satellite cell availability prevents muscle hypertrophy against weight loading in mice [6], suggesting its role in muscle plasticity against exercise challenges [4, 7, 8]. Paired box transcription factor 7 (Pax7) is a commonly used biomarker to identify satellite cells in muscle tissues in animal and human models [9–11]. While several studies have demonstrated increases in Pax7$^+$ cell content in exercised human skeletal muscle [12–15], the effect size and time required for a significant response after an acute bout of exercise from the pooled data of human studies have not been quantitatively examined.

One systematic review has first reported Pax7$^+$ cell expansion after a single bout of exercise [16]. Based on four original studies listed in the systematic review, satellite cells in human skeletal muscle increased incrementally and peaked at 72 h post-exercise [16]. More studies are required to delineate the magnitude of changes and time required for a significant increase during post-exercise recovery. It is generally observed that the degree of muscle hypertrophy induced by exercise attenuates with age [17]. This has been thought to be associated with reduced satellite cell reserves in muscle tissues [4, 18]. A quantitative analysis is required to confirm the effect size of post-exercise Pax7$^+$ cell expansion at a higher age. Furthermore, the results of Pax7$^+$ cell expansion after an acute bout of exercise between trained and untrained individuals remains inconclusive [7, 8, 19]. Differing exercise regimens may also influence Pax7$^+$ cell numbers of challenged human skeletal muscle [4]. Pax7$^+$ cells do not appear to increase in human muscle following a 6-week high-intensity aerobic training [20]. Subgroup analyses are needed to confirm the effects of age, post-exercise time, training status, and exercise regimen on changes of Pax7$^+$ cells in human skeletal muscle following an acute bout of exercise.

Bone marrow-derived multipotent stem cells help to maintain adequate stem cell reserves in muscle tissue for myofiber regeneration following damage [21–23]. Transplantation of CD34$^+$ bone marrow stem cells into damaged muscle tissues has been shown to increase satellite cells in murine muscles [23–25]. CD34 protein is a commonly used biomarker to identify multipotent bone marrow stem cells in circulation [26], and has been detected in some Pax7$^+$ cells of muscle tissues [27–32]. An acute bout of exercise increases CD34$^+$ cells surrounding myofibers in human skeletal muscles within 24 h [33, 34]. The purpose of this meta-analysis is to provide quantitative measures regarding the effect of a single-bout exercise on Pax7$^+$ cells in human skeletal muscle and to delineate the time course of exercise response in temporal relation with circulating CD34$^+$ cells during a 96-h recovery period. Subgroup analyses were also performed to confirm the effects of age, training status, and exercise regimen.

2 Methods

2.1 Study Protocol and Registration

The present work has been registered to the International Prospective Register for Systematic Reviews (PROSPERO, registration number: CRD42021265457).

2.2 Search Strategy

The literature search was performed for relevant studies (including a range of publications through to February 2022) across four databases: Web of Science, PubMed, Scopus, and BASE, according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guideline [35]. The keywords included: “exercise,” “Pax7,” “CD34,” “human skeletal muscle,” “satellite cells,” “endothelial progenitor cells,” and “progenitor cells”.

2.3 Inclusion/Exclusion Criteria

Studies were selected based on the PICOS model [36] (Table 1). Intervention studies conducting a single bout of aerobic and/or resistance exercise were included. The primary outcome of the present meta-analysis was $Pax7^+$ cells in human skeletal muscle. $CD34^+$ cells in circulation were a secondary outcome. Studies were excluded if they had: (1) undefined follow-up time after exercise; (2) mixed intervention (i.e., blood flow restriction combined with exercise); (3) unavailable total $Pax7^+$ cell counts; (4) the unavailable baseline data; and (5) no dispersion of dataset.

2.4 Data Extraction

The initial review records from all databases and the eligibility of studies were conducted by the primary investigator, then those results were confirmed by at least two separate investigators. The records were imported into Endnote (version 20.1; Clarivate Analytic, Philadelphia, PA, USA) and were automated and manually screened. Once the included studies were finalized, the data were categorized by the characteristics of participants (sample size, age, and sex), and the exercise modality. The outcome data were expressed as standardized mean difference (SMD). If the full-text article only presented in a figure format, WebPlotDigitizer (WebPlotDigitizer, Version 4.2, 2019; Ankit Rohatgi, TX, USA) was used to extract the data from the studies.

2.5 Data Analysis

Initially, a time analysis was conducted to distinguish the outcomes based on post-exercise skeletal muscle biopsy timepoints for < 24 h and ≥ 24 h and discovered a varying effect size with recovery time. Therefore, a subgroup analysis was further adapted by distinguishing multiple time categories. The exercise regimen included aerobic exercise and resistance exercise for comparison. Aerobic exercise predominated by concentric contraction was included [37]. We excluded a downhill running study from the meta-analysis assessing the effect of aerobic exercise due to a potentially greater muscle damage induced by eccentric muscle contraction [38]. For aerobic exercise, we categorized the intensity into moderate and high. High intensity was defined by either the running speed/cycling work rate as $\geq 77\%$ of heart rate $(HR)_{max/peak}$ or 60–90% of heart rate reserve or $\geq 80\%$ of maximum oxygen consumption (VO_{2max}) or $\geq$ anaerobic threshold. Moderate intensity of aerobic exercise was determined as 64–76% of heart rate maximum/peak or 40–59% of heart rate reserve or 46–79% of $VO2_{max}$. The studies using peak work rate were converted into %HR for which 59.5% of peak work rate corresponds to approximately 77% heart rate maximum [39]. Intensity of resistance exercise was classified into low ($< 50\%$ 1RM), moderate (50–69% 1RM), and high ($\geq 70\%$ 1RM) [40, 41]. We classified the training status into untrained and trained individuals based on the terminology used in previous work [42]. Untrained individuals were defined as subjects who participated in physical activity less than 3 h/week, while the trained subjects were defined as having habitual physical activity approximately 2 h/day for at least 3 days/week.

2.6 Quality Assessment

The quality assessment of the included studies comprised five domains according to the revised Cochrane Risk of Bias tool for exercise intervention trials: (1) randomization process; (2) deviations from intended interventions; (3) missing outcome data; (4) measurement of the outcome; and (5) selection of the reported result. The overall risk of bias was defined as "low risk" if all domains were at low risk of bias, "some concerns" if containing at least one domain at some concerns status, but not at high risk of bias for any domain,

Table 1 PICOS model used to perform the meta-analysis

Parameter	Inclusion criteria
Population	Healthy individuals (BMI: 18–25 kg/m^2)
Intervention	1. Aerobic exercise (acute bout of cycling or treadmill) with intensity $\geq 60\%$ VO_{2max} 2. Resistance exercise (acute bout) included all muscle contraction involved
Comparators	Pre-exercise baseline measurements
Outcomes	1. Primary outcome: $Pax7^+$ cells in biopsied muscle measured by an immunohistochemical analysis 2. Secondary outcome: circulating CD34+ cells including total $CD34^+$ cells, $CD34^+$/CD45dim, $CD34^+/CD45^+$, $CD34^+/Cd45^{dim}/VEGRF2^+$, in the circulation measured by a flow-cytometric analysis
Study design	Randomized and non-randomized trial

BMI body mass index, *CD34* cluster of differentiation 34 (commonly used to assess hematopoietic stem cells in blood), *PAX7* paired box transcription factor 7 (commonly used to assess muscle stem cells in tissues), VO_{2max} maximum oxygen consumption

and "high risk" if at least one study was judged in some concerns for multiple domains [43].

2.7 Statistical Analysis

This meta-analysis was performed using Review Manager (RevMan Version 5.4.1; The Cochrane Centre, Oxford, UK). We collected the baseline and post-exercise data of mean, standard error (SE), and sample size of individuals from exercise intervention studies. Forest plot was produced to display SMD, SE, and overall effect of Z score. If publications reported standard deviation (SD) only, SE was calculated using the following formula, where n represented the number of participants:

$$\mathrm{SE} = \frac{\mathrm{SD}}{\sqrt{n}}.$$

A random-effect model was used assuming an existence of inherent heterogeneity of the data among studies. To perform the SMD, we used the formula according to Cochrane [44]:

$$\mathrm{SMD} = \frac{\mathrm{mean}_{\mathrm{post}} - \mathrm{mean}_{\mathrm{baseline}}}{\frac{\mathrm{SD}_{\mathrm{paired}}}{\sqrt{2x(1-r)}}}.$$

The effect size was categorized into: (1) small (SMD = 0.20–0.50); (2) medium (SMD = 0.51–0.80); and (3) large (SMD > 0.8) [45–47]. Standard deviation values were calculated by:

$$\mathrm{SDp} = \sqrt{(\mathrm{SD}_{\mathrm{baseline}})^2 + (\mathrm{SD}_{\mathrm{post}})^2 - 2 \times r \times \mathrm{SD}_{\mathrm{baseline}} \times \mathrm{SD}_{\mathrm{post}}},$$

where r represents the correlation coefficient. The 95% confidence interval including "0" referred to non-statistically significant [48]. A positive effect of exercise into $Pax7^+$ cells and $CD34^+$ cells were pointed out by a positive SMD. A negative SMD showed the negative effect of exercise towards $Pax7^+$ cells and $CD34^+$ cells. The overall effect size using the Z-score was considered as significant at $p < 0.05$.

To assess the heterogeneity, tau-squared (τ^2), Chi-square Cochran's Q (χ^2) test, and I^2 statistic were performed. The value of $\tau^2 > 1$ indicated variability between studies. The Q test measured the variation around a weighted mean, in which a p value < 0.10 was considered as significant heterogeneity [49]. The I^2 statistic was used to assess the effect consistency across the studies, with the interpretation of I^2 as follows: (1) I^2 = 0–30% showing no important heterogeneity; (2) I^2 = 30–49% showing moderate heterogeneity; (3) I^2 = 50–74% showing substantial heterogeneity; and (4) I^2 = 75–100% showing considerable heterogeneity [44, 50].

3 Results

3.1 Literature Search

3.1.1 Selection Process

The number of identified articles from four databases and selection process are shown in Fig. 1. A total of 1719 intervention studies were retrieved from the database search, and 568 duplicated and ineligible articles were excluded. The screening phase in this work, including title and abstract screening, left 51 articles. The authors excluded five articles from the meta-analysis because of: (1) one mixed intervention study with whole-body vibration [51]; (2) two studies without a mean or SD [52, 53]; (3) one study presenting a $Pax7^+/MyoD^+$ sub-fraction with no total $Pax7^+$ data [4]; and (4) one study using aerobic running in the eccentric contraction mode [38]. These studies were included in the systemic review. This screening resulted in 46 eligible articles that were used for the current quantitative analysis, including 20 studies (n = 370) comprising $Pax7^+$ cell assessments in human skeletal muscle and 26 studies (n = 494) of circulating $CD34^+$ cell assessments in response to an acute bout of exercise.

3.1.2 Quality Assessment in Individual Studies

Among the included studies, no study scored in the high-risk bias, 12 studies scored in the moderate-risk bias [4, 12, 13, 15, 54–61], and 34 studies scored in the low-risk bias [7, 14, 16, 59, 62–89]. Results of the quality assessment are shown in Table S1 of the Electronic Supplementary Material (ESM).

3.2 Acute Response in Muscle $Pax7^+$ Cell Content After Exercise

3.2.1 Age and Training Status

Table 2 summarizes the sample size, age, exercise type, follow-up time, and $Pax7^+$ cell change from the pre-exercise baseline (%) in human skeletal muscle. The total number of participants were 370 (male/female = 360/10) with an age range of 21–74 years. The subgroup analysis presents the acute exercise response of muscle $Pax7^+$ cell content compared with the pre-exercise baseline for two age levels: ≤ 50 years and > 50 years (Fig. S1 of the ESM). A total of eight studies consisting of 105 participants aged > 50 years reported changes of muscle $Pax7^+$ cell content from the pre-exercise baseline [7, 8, 19, 64, 73, 76, 90, 91]. For the subgroup analysis, a large effect in the adults age > 50 years (SMD = 0.81, 95% CI 0.48–1.14, I^2 = 60%,

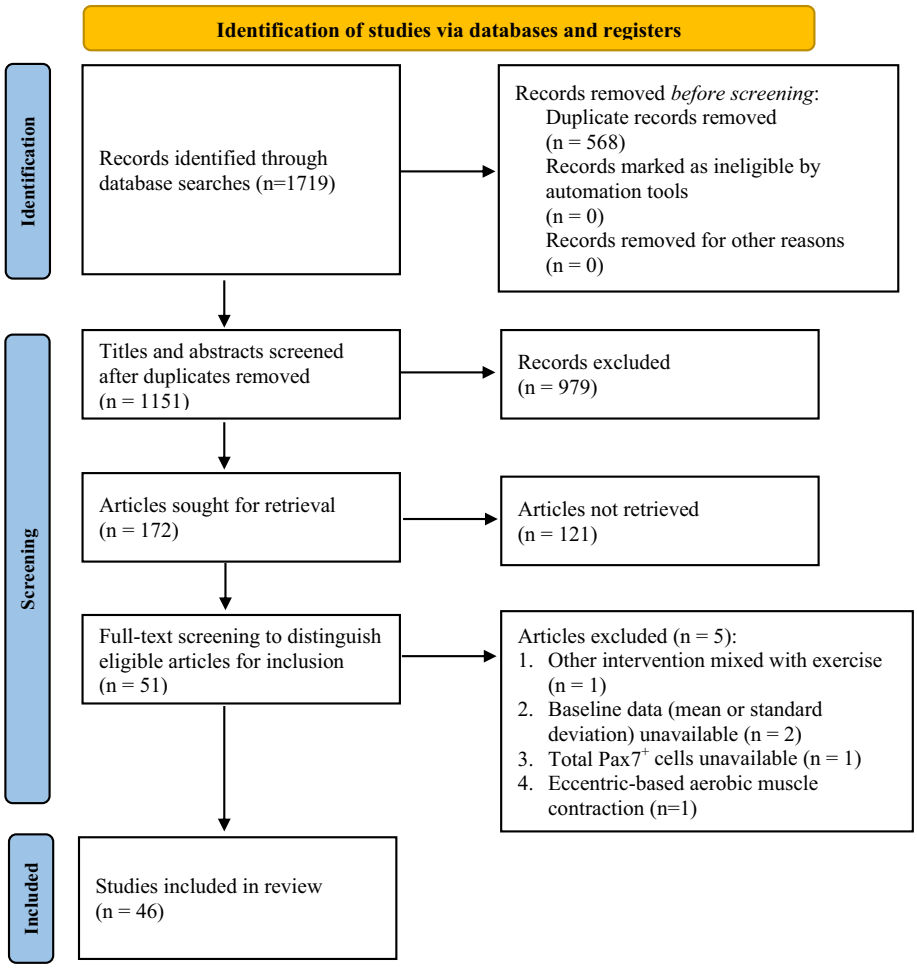


Fig. 1 Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) flow diagram outlining the electronic search and selection process

overall effect: $Z = 4.83$, $p < 0.001$) and a medium effect in the younger adults of Pax7$^+$ cell increases (SMD = 0.64, 95% CI 0.43–0.84, $I^2 = 66\%$, overall effect: $Z = 6.15$, $p < 0.001$) were observed. Figure S2 of the ESM shows the influence of training status of the participants for the acute exercise response in muscle Pax7$^+$ cell content. There were 12 studies recruiting untrained participants [7, 8, 14, 64–67, 73, 76, 90, 91] and eight studies recruiting trained participants [12, 13, 15, 19, 57, 63, 77, 90]. A large effect of exercise-induced Pax7$^+$ cell expansion was observed in the untrained subgroup regardless of age (SMD 0.81, 95% CI 0.61–1.02, $I^2 = 68\%$, overall effect: $Z = 7.79$, $p < 0.001$), whereas the trained subgroup showed a small effect (SMD 0.32, 95% CI 0.02–0.62, $I^2 = 52\%$, overall effect: $Z = 2.10$, $p < 0.05$).

3.2.2 Exercise Regimen

Most eligible studies assessing acute response in the Pax7$^+$ cell number of human muscle tissues used resistance exercise [7, 8, 12–15, 19, 57, 64–67, 73, 76, 77, 90–92]. Only one study reported acute response during and after concentric-based aerobic exercise [63]. The post-exercise follow-up time was limited to 96 h. Because of variations in exercise protocols (isokinetic eccentric contraction or weightlifting) used among the 18 resistance exercise studies, the effect of exercise intensity could not be accurately classified.

When all eligible studies of aerobic exercise and resistance exercise were included, data from 68 biopsied muscle samples (Fig. S3 of the ESM) showed a medium overall effect of post-exercise Pax7$^+$ cell increases in skeletal muscle (SMD = 0.68, 95% CI 0.51–0.86, $I^2 = 66\%$, overall effect: $Z = 7.74$, $p < 0.001$). The subgroup analysis showed no effect in Pax7$^+$ cell content to an acute bout of aerobic exercise (SMD = −1.03, 95% CI −2.76 to 0.70, $I^2 = 86\%$, overall effect: $Z = 1.17$, $p = 0.24$) (Fig. S3 of the ESM). A time analysis further showed an acute Pax7$^+$ cell depletion in muscle immediately after aerobic exercise followed by a quick return to baseline 3 h post-exercise (Fig. 2). No study assessing biopsied muscle immediately after resistance exercise was reported. When post-exercise recovery time was not considered, a medium effect of resistance exercise on Pax7$^+$ cell increases in skeletal muscles was observed (SMD = 0.73, 95% CI 0.56–0.89, $I^2 = 61\%$, overall effect: $Z = 8.63$, $p < 0.001$)(Fig. 3a). For the time analysis, the

Table 2 Characteristics of the studies included in this review of $Pax7^+$ cells in skeletal muscle studies with single-bout resistance exercise (**a**) and aerobic exercise (**b**)

Study, year	Biomarker	Sample size/age	Training status	Type (intensity)	Follow-up time post-exercise	Marker change from pre-exercise baseline
(a) Resistance exercise						
Bellamy et al., 2014 [12]	$Pax7^+$	Male ($n=23$), aged 24 y	Trained	Resistance exercise (acute, 80% of 1RM, 4 sets, 8 reps)	24 h	+34%
					72 h	+50%
Cermak et al., 2012 [77]	$Pax7^+$	Male ($n=9$), aged 23 y	Trained	15 sets, 20 reps at a speed 0.52 rad/s	24 h	+19.8%
Dreyer et al., 2006 [7]	$Pax7^+$	Male ($n=10$), aged 21–35 y	Untrained	Resistance exercise (acute, 6 sets; 12–16 reps at 60°/s)	24 h	+157%
	$Pax7^+$	Male ($n=9$), aged 60 y			24 h	+43%
Farup et al., 2014 [13]	$Pax7^+$	Male ($n=24$), aged 24 y	Trained	Resistance exercise (acute, 15 sets, 10 reps with range of motion were set at 70° and contraction velocity at 30°/s)	24 h	+19%
					48 h	0%
Hyldahl et al., 2014 [14]	$Pax7^+$	Male ($n=7$), aged 22 y	Untrained	Resistance exercise dominated by eccentric contraction (acute, achieved up to 40 kJ)	24 h	+28%
	$Pax7^+$	Male ($n=7$), aged 23 y		Resistance exercise dominated by concentric contraction (acute, achieved up to 40 kJ)		+3%
McKay et al., 2010 [100]	$Pax7^+$	Male ($n=12$), aged 21 y	Untrained	30 sets of 10 maximal muscle lengthening contractions at 3.14 rads/s	24 h	+37.9%
McKay et al., 2012 [76]	$Pax7^+$	Male ($n=9$), aged 21 y	Untrained	Resistance exercise (acute, 75% of 1-RM, 4 sets, 10 reps)	24 h	+32%
					48 h	+44%
		Male ($n=9$), aged 70 y			24 h	+21%
					48 h	+5%
McKay et al., 2013 [73]	$Pax7^+$	Male ($n=9$), aged 21.3 y	Untrained	Resistance exercise (acute, 75% of 1 RM, 4 sets, 10 reps)	3 h	+6%
					24 h	+22%
					48 h	+34%
		Male ($n=9$), aged 69.6 y			3 h	−1%
					24 h	+15%
					48 h	+29%
Mackey et al., 2016 [66]	$Pax7^+$	Male ($n=14$), aged 21 y	Untrained	Resistance exercise (acute, 5 sets, 20 reps with range of motion from 90 to 10°)	2.5 h	−2%
					48 h	−13%
Nederveen et al., 2015 [4]	$Pax7^+MyoD^+$	Male ($n=7$), aged 67 y	Untrained	Resistance exercise (acute, 95% of 10-RM, 4 sets, 10 reps for leg extension, 19 reps for leg press)	24 h	+269%
					48 h	+5%
	$Pax7^+MyoD^-$				24 h	−19%
					48 h	−18%
Nederveen et al., 2016 [15]	$Pax7^+$	Male ($n=23$), aged 24 y	Trained	Resistance exercise (acute, 80% of 1 RM, 4 sets, 8 reps)	24 h	+25%
					72 h	+31%

Table 2 (continued)

Study, year	Biomarker	Sample size/age	Training status	Type (intensity)	Follow-up time post-exercise	Marker change from pre-exercise baseline
Nederveen et al., 2017 [57]	$Pax7^+$	Male ($n=14$), aged 25 y	Trained	Resistance exercise (acute, 70–85% of 1 RM, 4 sets, 8 reps)	24 h	+4%
					72 h	+32%
Nederveen et al., 2018 [67]	$Pax7^+$	Male ($n=20$), aged 21 y	Untrained	Resistance exercise (acute, 30 sets, 10 reps at 180°/s, high CFPE)	6 h	+48
					24 h	+74%
					72	+57%
					96	+4%
				Resistance exercise (acute, 30 sets, 10 reps at 180°/s, low CFPE)	6 h	+38%
					24 h	+62%
					72	+47%
					96	+32%
Nederveen et al., 2020 [64]	$Pax7^+$	Male ($n=24$), Aged 73 y	Untrained	Resistance exercise (acute, 65% of 1 RM, 4 sets, 10 reps)	24 h	+61%
					48 h	+30%
Reidy et al., 2017 [91]	$Pax7^+$	Male ($n=19$), aged 70 y	Untrained	Resistance exercise (acute, 60–70% of 1RM, 8 sets, 10 reps)	1 h	−14%
Roberts et al., 2015 [53]	$Pax7^+$	Male ($n=10$), aged 22.1 y	Trained	Resistance exercise (acute, 8–12 RM) with ACT	2 h	+3%
					24 h	+20%
					48 h	+48%
				Resistance exercise (acute, 8–12 RM) with CWI	2 h	+1%
					24 h	+12%
					48 h	+16%
Snijders et al., 2014a [92]	$Pax7^+$	Male (n=20), aged 21 y	Untrained	Resistance exercise (acute, 75% of 1 RM, 6 sets, 10 reps) with LPD	12 h	+6%
					24 h	+17%
					48	+45%
					72	+57%
				Resistance exercise (acute, 75% of 1 RM, 6 sets, 10 reps) with NPD	12 h	+2%
					24 h	+16%
					48	+33%
					72	+42%
Snijders et al., 2014b [8]	$Pax7^+$	Male ($n=10$), aged 22 y	Untrained	Resistance exercise (acute, 75% of 1 RM, 6 sets, 10 reps)	12 h	+6%
					24 h	+26%
					48 h	+43%
					72 h	+53%
		Male ($n=10$), aged 73 y			12 h	+2%
					24 h	+14%
					48 h	+22%
					72 h	+31%
Snijders et al., 2019 [19]	$Pax7^+$	Male ($n=14$), aged 74 y	Untrained	Resistance exercise (acute, 65% of 1-RM, 4 sets, 10 reps)	24 h	+30%
					48 h	+5%
Toth et al., 2011 [65]	$Pax7^+$	Male ($n=12$)	Untrained	Resistance exercise (acute, 300 unilateral isokinetic eccentric contractions in 180°/s over a 55° range of motion)	1 h	+12%
					3 h	+17%
					24 h	+60%

Table 2 (continued)

Study, year	Biomarker	Sample size/age	Training status	Type (intensity)	Follow-up time post-exercise	Marker change from pre-exercise baseline
Walker et al., 2012 [19]	$Pax7^+$	Male ($n=5$), aged 27 y	Trained	Resistance exercise (acute, 70% of 1-RM, 8 sets, 10 reps)	6 h	+18%
					24 h	+138%
		Female ($n=5$), aged 27 y			6 h	+16%
					24 h	+50%
		Male ($n=6$), aged 70 y			6 h	+1%
					24 h	+48%
		Female ($n=5$), aged 70 y			6 h	+102%
					24 h	+140%
(b) Aerobic exercise						
Nederveen et al., 2015 [4]	$Pax7^+MyoD^+$	Male ($n=7$), aged 67 y	Untrained	Aerobic exercise (acute, 60% VO_2 peak)	24 h	106%
					48 h	70%
		Male ($n=8$), aged 67 y		Aerobic exercise (acute, 90–95% VO_2 peak)	24 h	355%
					48 h	51%
	$Pax7^+/MyoD^-$	Male ($n=7$), aged 67 y		Aerobic exercise (acute, 60% VO_2 peak)	24 h	−6%
					48 h	−33%
		Male ($n=8$), aged 67 y		Aerobic exercise (acute, 90–95% VO_2 peak)	24 h	−39%
					48 h	−12%
van De Vyver and Myburgh, 2012 [38]	$Pax7^+$	Male ($n=6$), aged 22 y	Untrained	Aerobic exercise Resistance exercise (acute, 85% VO_{2max} downhill running)	24 h	+41%
					48 h	+6%
Wu et al., 2019 [63]	$Pax7^+$	Male ($n=12$), aged 21 y	Trained	Aerobic exercise (acute, 70% VO_{2max})	0 h	−32%
					3 h	−3%

ACT active recovery, *CWI* cold water immersion, *h* hours, *LPD* low-protein diet, *NPD* normal protein diet, *RM* repetition maximum, *s* seconds, *y* years, *min* minutes, VO_{2max} maximum oxygen consumption maximum

effect size of resistance exercise peaked at 24 h and gradually returned to baseline in 96 h (Fig. 3b). The subgroup analysis showed a minimal effect within 12 h following resistance exercise (SMD = 0.22, 95% CI 0.01–0.42, $I^2 = 0\%$, overall effect: $Z = 2.09$, $p < 0.05$). A large effect of $Pax7^+$ cell increases after resistance exercise was contributed mostly by the muscle data assessed 24 h post-exercise or later (SMD = 0.89, 95% CI 0.64–1.14, $I^2 = 55\%$, overall effect: $Z = 6.95$, $p < 0.001$).

3.3 Acute Response in Circulating $CD34^+$ Bone Marrow Stem Cells After Exercise

$CD34^+$ bone marrow-derived stem cells from circulation contributes to local $Pax7^+$ cell reserve and myogenesis in skeletal muscle [27–32]. In this study, we also assessed the temporal relationship between circulating blood $CD34^+$ cells and $Pax7^+$ cell expansion in skeletal muscle in response to an acute bout of exercise.

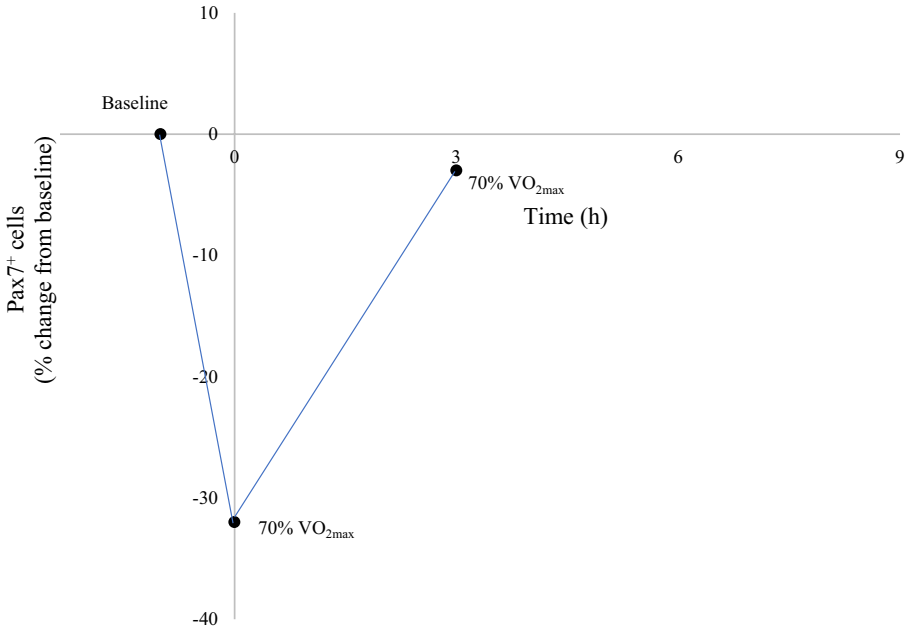


Fig. 2 Time course analysis for $Pax7^+$ cells in skeletal muscle after an acute bout of aerobic exercise with moderate intensity, 70% maximum oxygen consumption (VO_{2max})

3.3.1 Age and Training Status

Table 3 summarizes sample size, age, exercise type, follow-up time, and marker change from the pre-exercise baseline (%) for $CD34^+$ cells in blood. The age range of participants was 10–65 years ($n = 494$) [54–56, 58–62, 68, 70–72, 75, 78–89, 93]. No significant effect for circulating $CD34^+$ cells was found after a single bout of exercise from 24 participants aged 50–65 years (SMD = 0.30, 95% CI − 0.31 to 0.91, $I^2 = 62\%$, overall effect: $Z = 0.97$, $p = 0.33$). A medium effect was found for the remaining 470 young participants (SMD = 0.53, 95% CI 0.40–0.66, $I^2 = 69\%$, overall effect: $Z = 7.83$, $p < 0.001$) (Fig. S4 of the ESM). The effect of training status is shown in Fig. S5 of the ESM, which included (male/female = 378/116) categorized as untrained participants in eight studies [59–62, 79, 82, 85, 88] and trained participants in 19 studies [54–56, 58, 62, 68–70, 72, 75, 78, 80, 81, 83, 84, 86–89]. The subgroup analysis showed a medium effect for untrained (SMD = 0.57, 95% CI 0.38–0.77, $I^2 = 53\%$, overall effect: $Z = 5.82$, $p < 0.001$) and a small effect for trained participants (SMD = 0.49, 95% CI 0.32–0.67, $I^2 = 74\%$, overall effect: $Z = 5.58$, $p < 0.001$) compared with pre-exercise circulating $CD34^+$ cells.

3.3.2 Exercise Regimen

A total of 22 eligible studies for aerobic exercise [54–56, 58, 59, 61, 62, 68, 69, 71, 72, 75, 78–83, 85, 87–89] and five eligible studies for resistance exercise [60, 70, 82, 84, 86] assessing circulating $CD34^+$ cell counts were included in the quantitative analysis. The follow-up time after aerobic and resistance exercise was no more than 96 h. Without considering time after exercise, the overall effect of a single bout of aerobic exercise and resistance exercise based on 125 measurements (Fig. S6 of the ESM) showed a small and medium effect of exercise on increasing circulating $CD34^+$ cells, respectively (aerobic exercise: SMD = 0.47, 95% CI 0.32–0.63, $I^2 = 71\%$, overall effect: $Z = 6.17$, $p < 0.001$; resistance exercise: SMD = 0.67, 95% CI 0.42–0.92, $I^2 = 60\%$, overall effect: $Z = 5.19$, $p < 0.001$).

When post-exercise time was not considered, a small effect of aerobic exercise (SMD = 0.47, 95% CI 0.32–0.63, $I^2 = 71\%$, overall effect: $Z = 6.17$, $p < 0.001$) and a medium effect of resistance exercise for increasing circulating $CD34^+$ cells (SMD = 0.67, 95% CI 0.42–0.92, $I^2 = 60\%$, overall effect: $Z = 5.19$, $p < 0.001$) were observed. The time analysis has further shown immediate increases of circulating $CD34^+$ cells above baseline after aerobic exercise followed by a quick decline within 2 h post-exercise (Fig. 4a). A medium effect in circulating $CD34^+$ cell increases within 2 h after aerobic exercise was observed (SMD = 0.60, 95% CI 0.41–0.79, $I^2 = 73\%$, overall effect: $Z = 6.28$, $p < 0.001$) (Fig. 4b), whereas no significant effect was observed ≥ 2 h post-exercise (SMD = 0.18, 95% CI − 0.04 to 0.40, $I^2 = 52\%$, overall effect: $Z = 1.60$, $p = 0.11$). Both moderate-intensity aerobic exercise (46–79% VO_{2max} with average duration range of 10–240 min) [SMD = 0.48, 95% CI 0.24–0.72, $I^2 = 74\%$, overall effect: $Z = 3.90$, $p < 0.001$] and high-intensity aerobic exercise (≥ 80% VO_{2max} with average duration range of 6–207 min) [SMD = 0.47, 95% CI 0.28–0.67, $I^2 = 68\%$, overall effect: $Z = 4.74$, $p < 0.001$] showed a small effect on post-exercise increases in circulating $CD34^+$ cells (Fig. 4b).

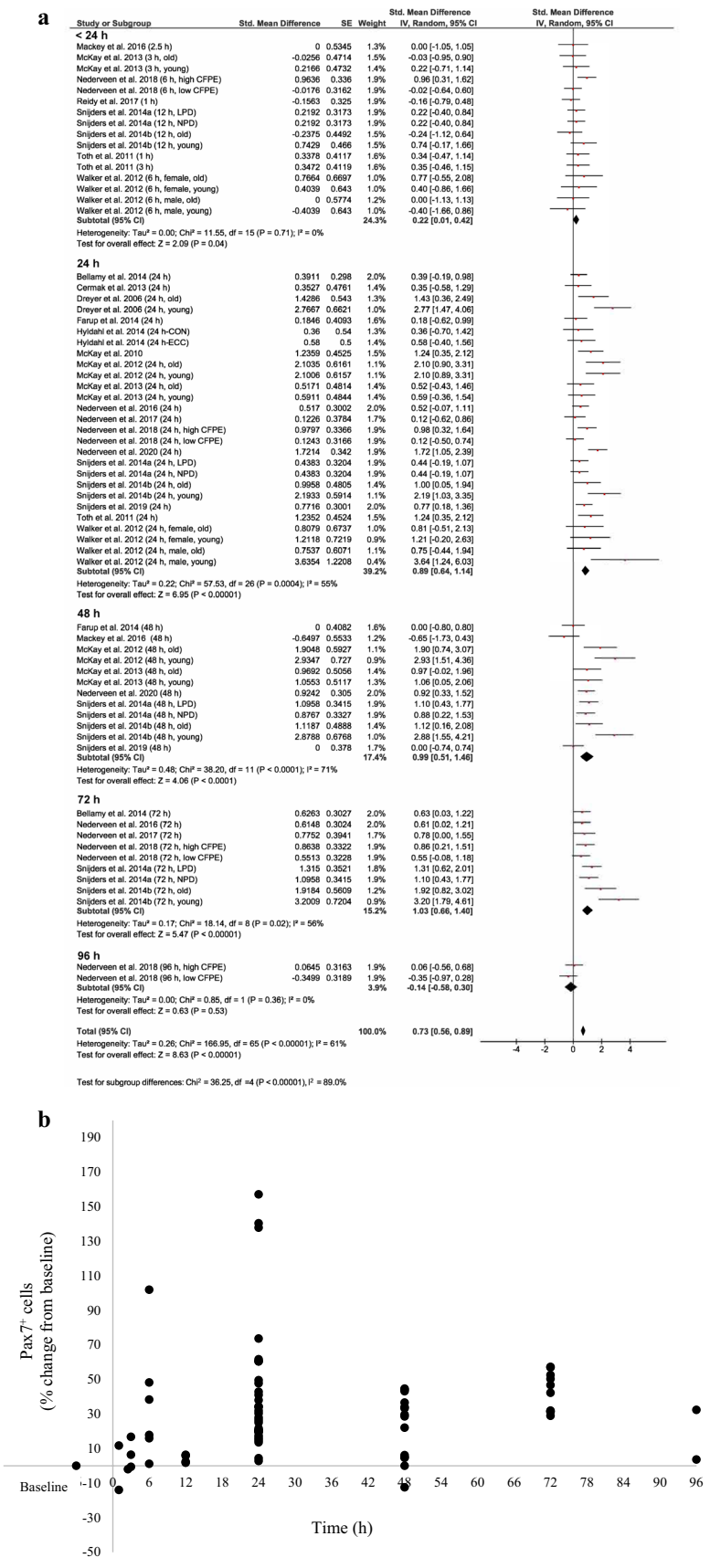


Fig. 3 Forest plot of standardized mean difference for $Pax7^+$ cell number in human skeletal muscle at follow-up measurement < 24 h, 24 h, 48 h, 72 h, and 96 h after resistance exercise (**a**), time course analysis for the change of $Pax7^+$ cells in skeletal muscle after an acute bout of resistance exercise (**b**). *CI* confidence interval, *df* degree of freedom, I^2 inconsistency between studies, *SE* standard error

Table 3 Summary and characteristics of the studies included in this review of circulating $CD34^+$ cells studies with single-bout resistance exercise (**a**) and aerobic exercise (**b**)

Study, year	Biomarker	Sample size	Training status	Type (intensity)	Follow-up time post-exercise	Marker change from baseline
(a) Resistance exercise						
Krüger et al., 2015 [82]	$CD34^+CD45^+$	Male ($n=12$), aged 25–26 y	Untrained	Resistance exercise (acute, 75% 1RM)	0 h	+70%
					3 h	+81%
					24 h	+51%
					48 h	−8%
	$CD34^+CD45^-KDR^+$				0 h	100%
					3 h	198%
					24 h	394%
					48 h	35%
Lee et al., 2015 [70]	$CD34^+$	Male ($n=6$), aged 28 y	Trained	Resistance exercise (acute, 10 sets; 6 reps MVC)	0 h	−3%
					2 h	−4%
					24 h	+19%
	$CD34^+$				48 h	+16%
					72 h	+14%
					96 h	+7%
Montgomery et al., 2019 [86]	$CD34^+CD45_{dim}$	Male ($n=9$), aged 21 y	Trained	Resistance exercise (acute, 1 set 30 reps, followed by 3 sets of 15 reps 20% 1RM)	0 h	−4%
					0.5 h	−6%
	$CD34^+VEGRF2^+$				0 h	116%
					0.5 h	112%
	$CD34^+CD45_{dim}VEGRF2$				0 h	96%
					0.5 h	140%
Ribeiro et al., 2017 [60]	$CD45_{dim}/VEGFR2^+/CD34^+$	Female ($n=13$), aged 20.7 y	Untrained	60% 1-RM, 3 sets, 12 reps	0 h	58%
					6 h	31%
					24 h	16%
		Female ($n=12$), aged 21 y		70% 1-RM, 3 sets, 12 reps	0 h	133%
					6 h	69%
					24 h	−35%
		Female ($n=13$), aged 20.9 y		80% 1-RM, 3 sets, 12 reps	0 h	135%
					6 h	222%
					24 h	10%
Ross et al., 2013 [84]	$CD34^+$	Male ($n=13$), aged 22 y	Trained	Resistance exercise (acute, 15 repetitions of six exercises-leg press, seated chest press, leg curl, lat pulldown, knee extension, and triceps pushdown)	0 h	+85%
					2 h	+65%
					24 h	−2%
(b) Aerobic exercise						
Agha et al., 2018 [78]	$CD34^+$	Male/female ($n=8/7$), aged = 28 y	Trained	Aerobic exercise (ventilatory threshold + 15%)	0 h	+76%
					1 h	-8%
					2 h	+8%
					3 h	+24%
				Aerobic exercise (ventilatory threshold − 5%)	0 h	+12%
					1 h	0%

Table 3 (continued)

Study, year	Biomarker	Sample size	Training status	Type (intensity)	Follow-up time post-exercise	Marker change from baseline
					2 h	−20%
					3 h	−8%
		Male/female (n = 10/2), aged = 30 y		Aerobic exercise (lactate thresh-old + 10%)	0 h	+91%
					1 h	+3%
Baker et al., 2017 [54]	$CD34^+$	Male (n = 11), aged 23.5 y	Trained	Aerobic exercise (acute, 70% WR_{peak})	0 min	+97%
					10 min	+1%
					30 min	+23%
					60 min	+9%
Bonsignore et al., 2002 [56]	$CD34^+$	Male (n = 16), aged 41.8 y	Trained	Half-marathon (acute)	0 h	−25%
					< 24 h	−58%
				Marathon (acute)	0 h	+20%
					< 24 h	−49%
Bonsignore et al., 2010 [55]	$CD34^+$	Male (n = 17), aged 43.6 y	Trained	Aerobic exercise (acute, 101% HR_{max})	0 h	+90%
				Aerobic exercise (acute, marathon)	0 h	−37%
					18 h	−17%
					24 h	+265%
Chang et al., 2015 [61]	$VEGFR2^+/CD11b^-/CD34^+/AC133^+$	Male (n = 5), aged 29.8 y	Untrained	Aerobic exercise (acute, HR > 140 bpm)	0 h	10%
					24 h	265%
Craenenbroeck et al., 2008 [71]	$CD34^+$	Male/female (n = 6/5), aged 23.9 y	Not reported	Aerobic exercise (acute, 116% $VO2_{max}$)	0 h	+39%
		Male/female (n = 9/6), aged 36.2 y		Aerobic exercise (acute, 119% $VO2_{max}$)	0 h	+10%
Harris et al., 2017 [79]	$CD34^+$	Female (n = 15), aged 63 y	Untrained	Aerobic exercise (acute, moderate continues, 80% of lactate threshold)	0.5 h	−19%
		Female (n = 15), aged 63 y		Aerobic exercise (acute, moderate interval, 90% of VO_{2max} with 10:20 s)	0.5 h	−4%
		Female (n = 15), aged 63 y		Aerobic exercise (acute, heavy interval, 90% of VO_{2max} with 30:60 s)	0.5 h	−15%
Kroepfl et al., 2012 [81]	$CD34^+$	Male (n = 10) aged 25.3 y	Trained	Aerobic exercise (acute, 40-W starting load, increasing 20 W/min) until exhaustion)	10 min	+87%
					30 min	+8%
					60 min	+1%

Table 3 (continued)

Study, year	Biomarker	Sample size	Training status	Type (intensity)	Follow-up time post-exercise	Marker change from baseline
					120 min	−10%
Krüger et al., 2014 [82]	$CD34^+/CD45^+$	Male ($n=12$), aged 25–26 y	Untrained	Aerobic exercise (acute, CET, 80% VO_{2max})	0 h	+96%
					3 h	+53%
					24 h	+34%
		Male ($n=12$), aged 25–26 y		Aerobic exercise (acute, ECC,80% VO_{2max} with the run down 12%)	0 h	+77%
					3 h	+33%
					24 h	+60%
					48 h	+5%
	$CD34^+CD45^-KDR^+$	Male ($n=12$), aged 25–26 y		Aerobic exercise (acute, CET, 80% VO_{2max})	0 h	72%
					3 h	16%
					24 h	−42%
		Male ($n=12$), aged 25–26 y		Aerobic exercise (acute, ECC, 80% VO_{2max} with the run down 12%)	0 h	164%
					3 h	−14%
					24 h	5%
					48 h	−14%
Kröpfl et al., 2020 [89]	$CD34^+$	Male ($n=21$), aged 29–30 y	Trained	Aerobic exercise (acute, 85% of speed/power)	0 h	+17%
Laufs et al., 2005 [83]	$CD34^+CD133^+$	Male ($n=25$), aged 24.8 y	Trained	Aerobic exercise (acute, 82% VO_{2max})	0 h	+54%
				Aerobic exercise (acute, 68% VO_{2max}, 30 min)	0 h	+43%
				Aerobic exercise (acute, 68% VO_{2max}, 10 min)	0 h	+6%
	$CD34^+VEGRF2^+$			Aerobic exercise (acute, 82% VO_{2max})	0 h	120%
				Aerobic exercise (acute, 68% VO_{2max}, 30 min)	0 h	163%
				Aerobic exercise (acute, 68% VO_{2max}, 10 min)	0 h	6%
	$CD34^+CD117^+$			Aerobic exercise (acute, 82% VO_{2max})	0 h	34%
				Aerobic exercise (acute, 68% VO_{2max}, 30 min)	0 h	20%
				Aerobic exercise (acute, 68% VO_{2max}, 10 min)	0 h	5%

Table 3 (continued)

Study, year	Biomarker	Sample size	Training status	Type (intensity)	Follow-up time post-exercise	Marker change from baseline
Möbius-Winkler et al., 2009 [75]	$CD34^+$	Male ($n=18$), aged 32.4 y	Trained	Aerobic exercise (acute, 70% individual anabolic threshold)	0 min	+178%
					30 min	+99%
					1 h	+72%
					2 h	+94%
					24 h	+3%
Morici et al., 2005 [80]	$CD34^+$	Male/ female ($n=13/7$) aged 16–18 y	Trained	Rowing with average workload 322 W	0 min	+114%
Niemiro et al., 2017 [59]	$CD34^+$	Male ($n=7$), aged 25.3 y	Untrained	Aerobic exercise (acute, 70% VO_{2max})	15 min	+58%
					1 h	+5%
					2 h	+23%
O'Carroll et al., 2019 [58]	$CD34^+CD45d_{im}VEGRF2^+$	Male/female ($n=8/4$), aged 29 y	Trained	Aerobic exercise (acute, 70% VO_{2max})	0 h	+35%
					2 h	−19%
					24 h	−6%
	$CD34^+CD45_{dim}$				0 h	16%
					2 h	−191%
					24 h	3%
Ross et al., 2018 [93]	$CD34^+CD45_{dim}$	Male ($n=8$), aged 23 y	Trained	Aerobic exercise (acute, 70% VO_{2max})	0 h	+55%
		Male ($n=9$), aged 65 y			0 h	−2%
	$CD34^+CD45_{dim}VEGRF2^+$	Male ($n=8$), aged 23 y			0 h	104%
		Male ($n=9$), aged 65 y			0 h	62%
Shill et al., 2018 [68]	$CD34^+$	Male/female ($n=10/10$), aged 23.6 y	Trained	Aerobic exercise (acute, 65% VO_{2max})	0 min	+4%
					30 min	−18%
					1 h	−14%
					1.5 h	−23%
					2 h	−25%
				Aerobic exercise (acute, 90–100% VO_{2max})	0 min	0%
					30 min	−1%
					1 h	+7%
					1.5 h	+12%
					2 h	−1%
Stelzer et al., 2014 [131]	$CD34^+$	Male/female ($n=3/4$), aged 39.6 y	Trained	Ultra-endurance cycling race	0 min	−11%
Thijssen et al., 2006 [62]	$CD34^+$	Male ($n=8$), aged 19–28 y	Untrained	Aerobic exercise (acute, 65% HRR)	0 h	+100%
		Male (n=8), aged 19–28 y	Trained			+32%

Table 3 (continued)

Study, year	Biomarker	Sample size	Training status	Type (intensity)	Follow-up time post-exercise	Marker change from baseline
		Male ($n=4$), aged 67–76 y	Untrained			+71%
		Male ($n=4$), aged 67–76 y	Trained			+48%
Wardyn et al., 2008 [88]	$CD34^+$	Male/female ($n=10/8$), aged 19–35 y	Untrained	Aerobic exercise (acute, 90–112% VO_{2max})	30 min	0%
		Male/female ($n=9/10$), aged 19–35 y	Trained	Aerobic exercise (acute, 114–148% VO_{2max})	30 min	−3%
Yang et al., 2007 [85]	$CD34^+/KDR^+$	Male ($n=16$), Aged 25.1 y	Untrained	Aerobic exercise (acute, 5.5 km/h, 14% grade, 10.2 METS)	30 min	74%
Zaldivar et al., 2007 [72]	$CD34^+$	Male ($n=14$), aged 10.3 y	Trained	Aerobic exercise (acute, 50% WR)	0 h	+63%
		Male ($n=13$), aged 16.6 y			0 h	+141%

min minutes, *MVC* maximal voluntary contraction, *CET* concentric endurance test, *ECC* eccentric endurance test, *h* hour, HR_{max} heart rate maximum, *HRR* heart rate reserve, *s* seconds, VO_{2max} maximal oxygen consumption, *WR peak* work rate peak, *y* years

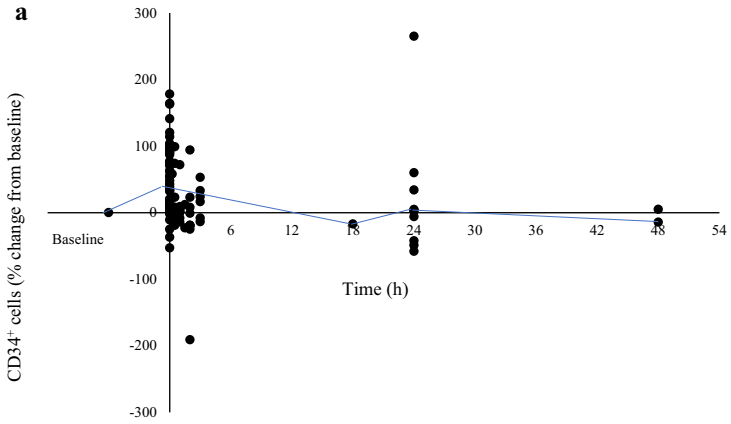
Resistance exercise showed immediate increases in circulating $CD34^+$ cells and returned to baseline levels after 6 h (Fig. 5a). A large effect of $CD34^+$ cell increases was observed within 6 h after resistance exercise (SMD = 0.93, 95% CI 0.60–1.27, $I^2 = 60\%$, overall effect: $Z = 5.45$, $p < 0.001$), whereas no significant effect was observed beyond 6 h (SMD = 0.29, 95% CI − 0.01 to 0.59, $I^2 = 33\%$, overall effect: $Z = 1.87$, $p = 0.06$) (Fig. 5b).

4 Discussion

To our knowledge, the present study provides the first quantitative meta-analysis to confirm the effect of an acute single-bout exercise on $Pax7^+$ cells in human skeletal muscles. Time required for significant changes in $Pax7^+$ cells during and after exercise is also delineated with circulating levels of $CD34^+$ cells [32, 94, 95]. Here, we summarized the findings as follows: (1) $Pax7^+$ cell replenishment and further expansion occur following a transient $Pax7^+$ cell depletion in human skeletal muscle during exercise; (2) $Pax7^+$ cell expansion in exercised human skeletal muscles peaks at 24 h and remains somewhat elevated up to 72 h after resistance exercise; (3) post-exercise $Pax7^+$ cell expansion occurs slowly after a transient increase in circulating $CD34^+$ bone marrow stem cells during exercise; and (4) $Pax7^+$ cell expansion induced by exercise remains normal for adults aged 50–74 years, despite a lower $Pax7^+$ cell reserve in muscle compared with younger adults.

4.1 $Pax7^+$ Cells in Human Skeletal Muscle Increases After Exercise in Higher Age Adults and Training Status

Lower $Pax7^+$ cell reserves in aging skeletal muscle are generally considered as a cause of age-dependent declines in muscle repair and regenerative capacity [96–98]. However, the current meta-analysis showed a slightly greater effect size of $Pax7^+$ cell increases after exercise in adults aged 50–74 years compared with younger adults (SMD = 0.81; $p < 0.001$ vs SMD = 0.64; $p < 0.001$). This unexpected result appears to be contributed in part by moderately lower baseline values of $Pax7^+$ cells in older than younger adults [18, 99]. The mean baseline value for older adults was 6.3 $Pax7^+$ cells/100 myofibers [4, 7, 19, 64, 73, 76, 90, 92] compared with an average of 9.9 $Pax7^+$ cells/100 myofibers in sedentary young adults [7, 8, 14, 15, 19, 57, 63, 66, 67, 73, 76, 77, 92, 100]. The larger effect size in older adults is mainly contributed by three studies [4, 64, 90]. Despite significant increases in $Pax7^+$ cell content after exercise for adults aged 50–74 years, the time required for this exercise response seems to be longer in those with a higher age according to four studies [7, 8, 19, 73]. The primary difference between these four studies and the other studies was from a delayed response in participants aged > 70 years. Anabolic hormones such as insulin and sex hormones are known to decline during late life [101–107], which may be responsible for this observation. Both sex hormones [108] and insulin [109] are essential for stem cell reproduction and tissue repair.



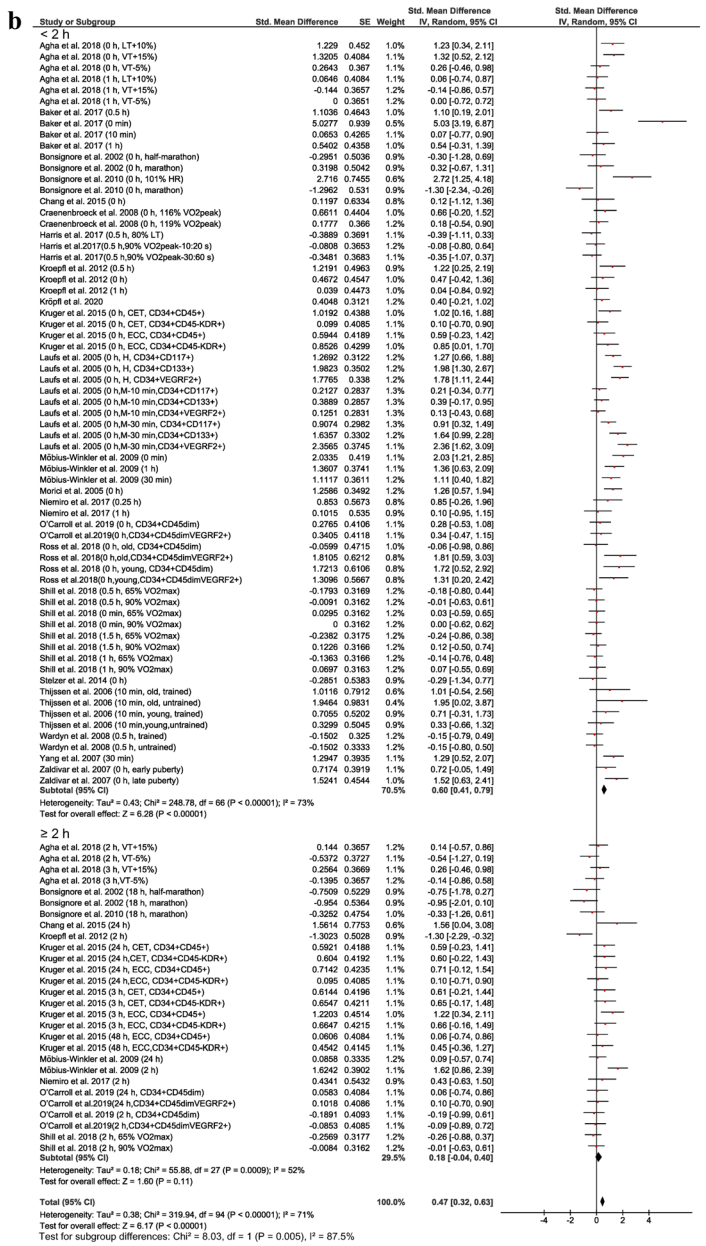


Fig. 4 Time course analysis of circulating $CD34^+$ cells following aerobic exercise (**a**), forest plot of standardized mean difference for $CD34^+$ cells in circulation at follow-up measurement < 2 h and ≥ 2 h after an acute bout of aerobic exercise (**b**). *CI* confidence interval, *df* degree of freedom, I^2 inconsistency between studies, *SE* standard error

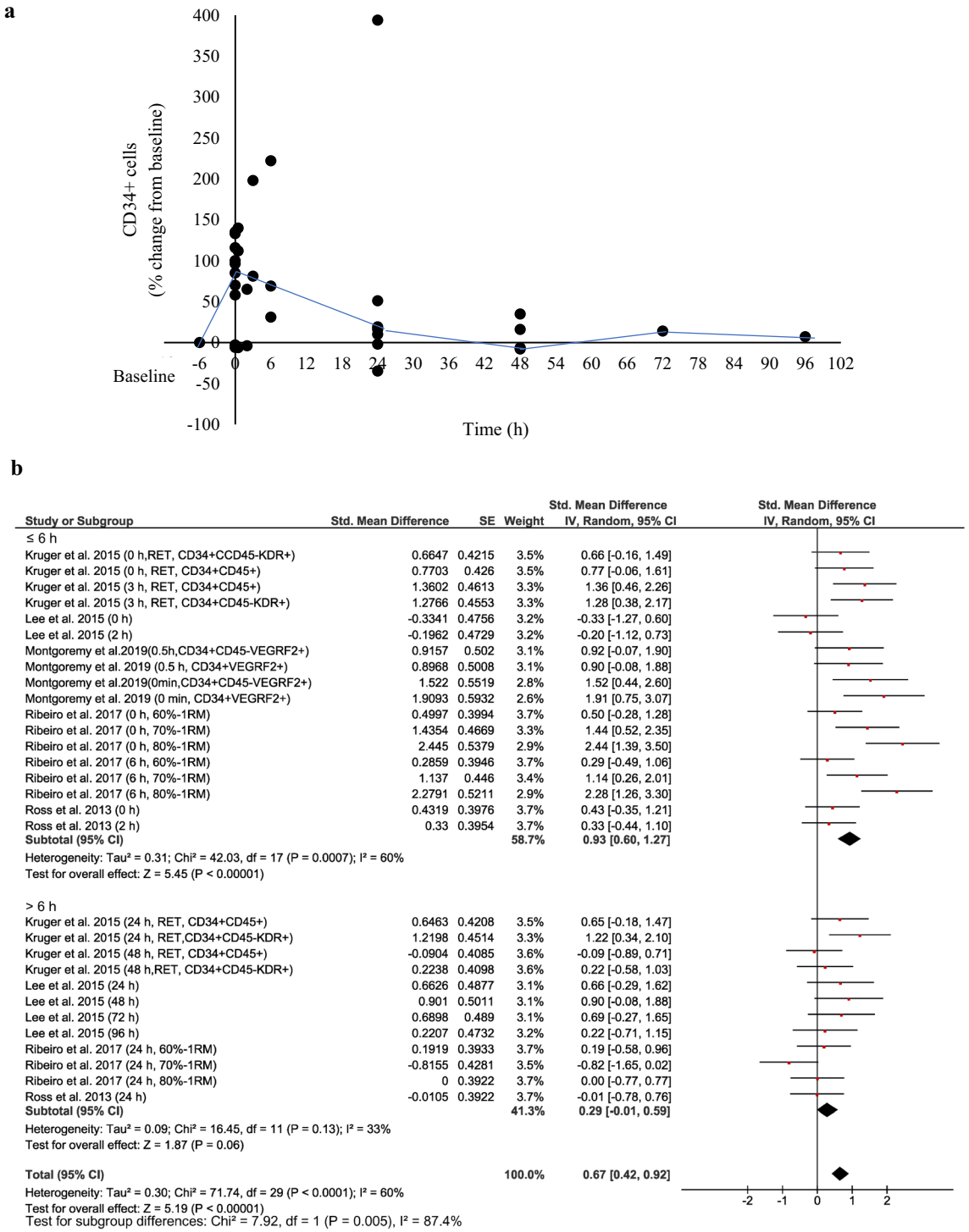


Fig. 5 Time course analysis on circulating $CD34^+$ cells following resistance exercise (**a**), forest plot of standardized mean difference for $CD34^+$ cells in circulation at follow-up measurement ≤ 6 h and > 6 h after resistance exercise (**b**). *CI* confidence interval, *df* degree of freedom, I^2 inconsistency between studies, *SE* standard error

The effect size of exercise on $Pax7^+$ cell expansion of human skeletal muscle is more pronounced in untrained than trained individuals (untrained: SMD = 0.81; $p < 0.001$ vs trained: SMD = 0.32; $p < 0.05$) (Fig. S2). This result suggests a greater level of inflammation occurs in untrained skeletal muscle during challenge. The causal relationship between $Pax7^+$ cell expansion and inflammation is supported by an increase in $Pax7^+$ cells after treatments of a mixture of

inflammatory mediators in an in vitro study [110]. The range of $Pax7^+$ cells of trained individuals was 3–33 cells per 100 myofibers [15, 19, 57, 63, 77] compared with 4–13 cells per 100 myofibers of the untrained individuals [4, 7, 8, 14, 38, 66, 67, 73, 76, 90, 92, 100]. There was only one exception that showed minimal changes (< 10%) in $Pax7^+$ cell increases 24 h following exercise with the low baseline value (11.5 cells per 100 myofibers) in active participants [57].

4.2 Aerobic Exercise-Induced Stem Cell Expansion in Human Muscle Remains Uncertain

The literature assessing the effect of aerobic exercise on $Pax7^+$ cell content is limited [4, 38, 63]. An acute bout of aerobic exercise was observed to transiently deplete $Pax7^+$ cells (− 32%) in human skeletal muscle followed by a rapid repletion in 3 h after a 60-min aerobic exercise at 70% VO_{2max} [63]. This brief reduction led to no overall effect in the current meta-analysis when the time of post-exercise recovery was not considered. One study showed an elevated $Pax7^+/MyoD^+$ fraction 24 h following high-intensity aerobic exercise at 90–95% VO_{2max} (+ 343%) and resistance exercise (+ 265%) concurrent with declines of $Pax7^+/MyoD^-$ fraction [4]. Moderate exercise at 55–60% VO_{2max} has no apparent effect on such changes. This study was excluded for the meta-analysis because the total $Pax7^+$ cell content was not provided.

Both aerobic and resistance exercises cause cell death [111, 112] and senescent cell clearance [34, 113] in muscle tissues, particularly at high intensity. $Pax7^+$ cells residing in surrounding myofibers are required for instantaneous donation of nuclei to maintain the size and youth of the muscle tissues [114, 115]. In this meta-analysis, resistance exercise shows more prominent response in post-exercise $Pax7^+$ cell increases compared with aerobic exercise. Despite no overall increases in muscle $Pax7^+$ cells after aerobic exercise, a significant increase in $Pax7^+$ cells (+ 41%) occurs when an aerobic exercise was conducted using eccentric muscle contraction [38]. Eccentric-based resistance exercise produces more muscle damage than concentric-based aerobic exercise [116]. In addition, aerobic exercise imposes a much greater challenge to pulmonary ventilation than resistance exercise, which inevitably causes lung damage and airway inflammation [117–119]. At rest, lungs are the main consumers of bone marrow-derived stem cells [120] for regenerating the short-lived airway epithelial cells [121]. Pulmonary illness is known to cause muscle loss [122, 123]. These findings point to a possibility that a competition for bone marrow-derived stem cells between lungs and muscles may explain the lower replenishment of $Pax7^+$ cells in muscle after aerobic exercise compared with resistance exercise.

4.3 $Pax7^+$ Cell Expansion in Human Muscle Peaks at 24 Hours and Vanishes by 96 Hours After Resistance Exercise

The present meta-analysis shows a large effect (SMD = 0.89, $p < 0.001$) of $Pax7^+$ cell expansion in response to resistance exercise, assessed 24 h after the workout. This significant effect can last for 72 h (SMD = 1.03, $p < 0.001$), which is consistent with a recent systematic review summarized from four original studies [16]. In this meta-analysis, we included 18 original resistance exercise studies that provided quantitative perspective of $Pax7^+$ cell expansion in human skeletal muscle, further suggesting that this response peaks at 24 h post-exercise. Resistance exercise induces a protracted $Pax7^+$ cell expansion in human skeletal muscle up to 157% in 24 h (SMD = 0.89, $p < 0.001$), 45% in 48 h (SMD = 0.99, $p < 0.001$), and 57% in 72 h (SMD = 1.03, $p < 0.001$) post-exercise. This effect vanished by day 4 (SMD = − 0.14, $p = 0.53$). $Pax7^+$ cells are essential for myogenesis [8, 12, 15, 57, 67, 92] following senescent cell clearance [34, 113], resulting in exercise-induced muscle rejuvenation [4, 124, 125]. However, a sustained $Pax7^+$ cell expansion also implies a longer period of inflammation. Inflammation is featured by pain, heat, redness, swelling, and loss of function which provide an explanation of delayed-onset muscle soreness after resistance exercise.

4.4 Exercise Induces a Transient Increase in Circulating $CD34^+$ Cells

$CD34^+$ bone marrow-derived stem cells are multipotent and can further differentiate into both endothelial progenitor cells and satellite cells in muscle tissues [32, 94, 95, 126]. The results of this study reveal a transient release of $CD34^+$ cells into circulation occurs prior to $Pax7^+$ cell expansion in human skeletal muscle. The response of increasing $CD34^+$ cells lasts no more than 2 h [55]. The temporal relationship of immediate increases of circulating $CD34^+$ cells followed by a delayed $Pax7^+$ cell expansion in muscle tissues suggests a replenishment of stem cell reserves in challenged muscle tissues from bone marrow. This result matches well to the observation of a transient depletion of $Pax7^+$ cells in skeletal muscle followed by a quick return to the pre-exercise level in 3 h [63], reflecting a quick migration of circulating bone marrow-derived hematopoietic stem cells into the challenged muscle tissues [55]. Both aerobic exercise [58, 68, 78, 79, 81, 88] and resistance exercise [82, 84] increase circulating $CD34^+$ cells to a similar extent. However, the rise-and-fall pattern of circulating $CD34^+$ cells appear to be delayed in an intensity-dependent manner during resistance exercise [60]. Taken together, a crosstalk between muscle and bone according to the magnitude of tissue inflammation might determine the duration of post-exercise release of circulating

$CD34^+$ cells and muscle $Pax7^+$ cell expansion. Circulating levels of $CD34^+$ cells is also influenced by the rate of bone marrow output and the rate of peripheral tissue consumption. As aforementioned, lungs are the major consumer of bone marrow stem cells [120, 127]. We cannot preclude the possibility that the quicker return to baseline of circulating $CD34^+$ cells after aerobic exercise is associated with greater stem cell demands from the challenged lungs compared with resistance exercise. In this meta-analysis, the insignificant result of exercise-induced circulating level of $CD34^+$ cells at higher age remains unclear [62, 69, 79]. It may be associated with greater inflammation and stem cell demand of lungs after exercise. Further analysis could include the CC16/SP-D ratio, a valid and sensitive marker for lung epithelium damage, assessed together with circulating levels of $CD34^+$ cells after exercise.

4.5 DNA Editing

The accumulated findings to date may be instrumental to indicate a new direction for improving muscle performance associated with genetic variation and aging. Techniques for enrichment and engraftment of autologous $CD34^+$ bone marrow stem cells following CRISPR-Cas9 DNA editing are currently available for humans [128, 129]. Recruitment of $CD34^+$ bone marrow stem cells to replenish peripheral stem cells in skeletal muscle requires tissue inflammation induced by exercise [130]. For those patients who have lose their capacity to exercise, contracting skeletal muscle via electrical stimulation can be an alternative way to induce adequate inflammatory response for homing and proliferating DNA-edited bone marrow stem cells. Exercise training combined with transplantation of DNA-edited $CD34^+$ bone marrow stem cells opens a vast range of future possibilities to engineer muscle phenotypes for both medical and non-medical purposes. This approach gives us a hope to accelerate evolution of humans into a species with better fitness.

5 Conclusions

$Pax7^+$ cells are myogenic stem cells contributing to muscle repair by a quick fusion of nuclei into the cytoplasm of myofibers and plays a key role in muscle plasticity against acute exercise challenges. $Pax7^+$ cells in muscle tissue are transiently depleted during exercise followed by a quick replenishment within 3 h, and further elevated (~ 50%) during 24–72 h after exercise. Exercise-induced $Pax7^+$ cell expansion in human skeletal muscle remains normal at higher age, despite lower reserves after age 50 years. Transient increases in circulating $CD34^+$ bone marrow stem cells during exercise are associated with post-exercise proliferation of $Pax7^+$ cells in exercised muscle. The immediate increases in circulating $CD34^+$ cells after both aerobic and resistance exercise suggest that inflammatory mediators originating from inflammation of damaged muscle may be responsible for triggering the release of bone marrow-derived stem cells and seeding to damaged muscle for repopulating muscle stem cells. More studies are required to conclude the effect of acute aerobic exercise on $Pax7^+$ cell content in human skeletal muscle.

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