Myonuclear permanence in skeletal muscle memory: a systematic review and meta-analysis of human and animal studies

Masoud Rahmati1*, John J. McCarthy2,3 & Fatemeh Malakoutinia1

1Department of Physical Education and Sport Sciences, Faculty of Literature and Human Sciences, Lorestan University, Khorramabad, Iran; 2Department of Physiology, University of Kentucky, Lexington, KY, USA; 3Center for Muscle Biology, University of Kentucky, Lexington, KY, USA

Abstract

One aspect of skeletal muscle memory is the ability of a previously trained muscle to hypertrophy more rapidly following a period of detraining. Although the molecular basis of muscle memory remains to be fully elucidated, one potential mechanism thought to mediate muscle memory is the permanent retention of myonuclei acquired during the initial phase of hypertrophic growth. However, myonuclear permanence is debated and would benefit from a meta-analysis to clarify the current state of the field for this important aspect of skeletal muscle plasticity. The objective of this study was to perform a meta-analysis to assess the permanence of myonuclei associated with changes in physical activity and ageing. When available, the abundance of satellite cells (SCs) was also considered given their potential influence on changes in myonuclear abundance. One hundred forty-seven peer-reviewed articles were identified for inclusion across five separate meta-analyses; (1–2) human and rodent studies assessed muscle response to hypertrophy; (3–4) human and rodent studies assessed muscle response to atrophy; and (5) human studies assessed muscle response with ageing. Skeletal muscle hypertrophy was associated with higher myonuclear content that was retained in rodents, but not humans, with atrophy (SMD = 0.60, 95% CI −1.71 to 0.51, P = 0.29, and MD = 83.46, 95% CI −649.41 to 816.32, P = 0.82; respectively). Myonuclear and SC content were both lower following atrophy in humans (MD = 11, 95% CI −0.19 to −0.03, P = 0.005, and SMD = 0.49, 95% CI −0.77 to −0.22, P = 0.0005; respectively), although the response in rodents was affected by the type of muscle under consideration and the mode of atrophy. Whereas rodent myonuclei were found to be more permanent regardless of the mode of atrophy, atrophy of ≥30% was associated with a reduction in myonuclear content (SMD = −1.02, 95% CI −1.53 to −0.51, P = 0.0001). In humans, sarcopenia was accompanied by a lower myonuclear and SC content (MD = 0.47, 95% CI 0.09 to 0.85, P = 0.02, and SMD = 0.78, 95% CI 0.37–1.19, P = 0.0002; respectively). The major finding from the present meta-analysis is that myonuclei are not permanent but are lost during periods of atrophy and with ageing. These findings do not support the concept of skeletal muscle memory based on the permanence of myonuclei and suggest other mechanisms, such as epigenetics, may have a more important role in mediating this aspect of skeletal muscle plasticity.

Keywords Muscle memory; Myonuclei; Satellite cell; Hypertrophy; Ageing; Meta-analysis

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*Correspondence to: Masoud Rahmati, Department of Physical Education and Sport Sciences, Faculty of Humanities, Lorestan University, Khorramabad, Iran.
Email: rahmati.mas@lu.ac.ir

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Introduction

Skeletal muscle fibres are some of the largest cells in the body and uniquely multinucleated with more than one hundred myonuclei per mm length of fibre. In order to maximize the distance between neighbouring nuclei, all nuclei within the syncytium are evenly positioned, adjacent to the plasma membrane. More interestingly, skeletal muscle is an extraordinary tissue with the ability to respond to intrinsic and extrinsic stimuli by changing its size. Myonuclei have an important role in skeletal muscle size adaptation through the production of transcripts that support the synthesis of proteins for use in the immediate vicinity surrounding each nucleus.

In response to exercise, new myonuclei can be acquired by myofibres as the result of fusion by muscle stem cells (known as satellite cells), which are normally in a quiescent state and become activated upon exposure to external stimuli, such as exercise or injury. Once activated, satellite cells (SCs) proliferate, differentiate into myogenic progenitor cells, and subsequently fuse to existing myofibres, providing additional nuclei to the growing myofibres. Studies have provided evidence showing that each nucleus within a myofibre oversees a given amount of cytoplasm, which is referred to as the myonuclear domain. The notion of a myonuclear domain is based on the concept that each nucleus has a limited capacity to control transcriptional characteristics over a finite volume of cytoplasm. Further, other studies have suggested the size of the myonuclear domain may not be as fixed as is often indicated.

Skeletal muscle possesses the remarkable ability to ‘recall’ a previous hypertrophic state upon resumption of training following a period of detraining, a phenomenon that has been called ‘muscle memory’. Scientists first attributed the phenomenon of muscle memory to motor learning via the central nervous system. The findings from more recent studies have proposed that muscle memory is related to the abundance of myonuclei, with the new myonuclei added during the initial hypertrophy being permanent, thereby providing enhanced transcriptional output in response to training following a bout of detraining. It has been hypothesized that the retention of the hyper-nucleated condition might be responsible for the accelerated regeneration and return of myofibre size and function even after a prolonged period of inactivity in previously trained skeletal muscle. Current available evidence regarding muscle memory is quite conflicting with some reports confirming myonuclear permanence, although other studies showing myonuclei could be lost during detraining. Some studies have reported that myonuclear content in skeletal muscle is not permanent and undergoes apoptosis with atrophy in response to hindlimb suspension, denervation, exposure to microgravity, and immobilization. Moreover, recent studies in both rodents and humans have shown that myonuclei acquired during hypertrophy are not permanent following long-term inactivity with myonuclear abundance returning to previously untrained state.

To the best of our knowledge, no systematic review and meta-analysis has yet assessed whether hypertrophy-induced myonuclear accretion is maintained after exercise cessation or inactivity in both humans and rodents. The aim of this systematic review and meta-analysis was to assess myonuclear and SC content in skeletal muscle that underwent hypertrophy or atrophy in both humans and rodents. Finally, the long-term myonuclear permanence in human was assessed by the inclusion of ageing studies in the meta-analyses.

Methods

The present preclinical and clinical review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the registration number: CRD42020152068 and was performed in accordance with PRISMA guidelines.

Research question

In the present systematic review and meta-analysis, we sought to answer the following questions: (i) Is hypertrophy-induced myonuclear accretion maintained after exercise cessation in either humans and/or rodents? (ii) Does myonuclear content and/or SC abundance change during atrophy in either humans or rodents? (iii) Is there any difference in myonuclear content and/or SC abundance between elderly and young adults?

Data sources and searches

A systematic literature search for relevant studies was carried out using the following databases: CINAHL, MEDLINE, CENTRAL, PEDro, ProQuest, and Scopus, from the earliest record of each database up to February 2022. Search terms included a combination of the following keywords related to muscle memory: ‘muscle memory’ and ‘memory’; related to muscle CSA: ‘muscle hypertrophy’, ‘muscle atrophy’, ‘myonuclei’, ‘myonuclear domain’, ‘satellite cell’, and ‘muscle stem cell’; related to training: ‘resistance exercise’, ‘resistance training’, ‘strength training’, ‘power training’, ‘endurance exercise’, and ‘endurance training’; related to atrophy stimuli: ‘loading’, ‘unloading’, ‘hindlimb suspension’, ‘suspension’, ‘leg immobilization’, ‘immobilization’, ‘step reduction’, ‘denervation’, ‘spinal cord injury’ and ‘spinal cord transaction’; and related to human ageing: ‘sarcopenia’, ‘human Aging’, ‘aging’, and ‘elderly’.
Study selection

We included all studies involving human and animal models independent of sex, age, and intervention (except steroid administration) that evaluated satellite cell or myonuclear abundance. In terms of study design, both controlled and uncontrolled clinical trials were included in the systematic review and meta-analysis (Figure 1).

Quality assessment

We assessed potential study bias using Physiotherapy Evidence Database (PEDro) scale for human studies by two independent researchers. All included human studies presented a score of ≤5.0. We also used the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) tool for assessing the risk of bias in animal studies. The results of quality assessments in both human and animal studies are outlined in Figure S1A–S1E.

Data extraction

Two reviewers independently (MR and FM) extracted all related information with disagreements between reviewers resolved by discussion. The included information was collected and organized into Tables 1–3. Information was extracted on study design characteristics (rodent species, sex, age, hypertrophy or atrophy model, etc.), type of intervention (training or atrophy duration), and outcome data (myonuclear content and satellite cell abundance). Included studies were grouped according to the following experiments: human subjects experienced hypertrophy, human subjects experienced atrophy, comparison of old vs. young people, animal models experienced hypertrophy, and animal models experienced atrophy.

Data analysis

All data analyses were conducted using Review Manager Software (RevMan 5.3, Cochrane Collaboration, Copenhagen, Denmark) as previously described in detail by us. For instance, when data was only available in a graphic format, we used WebPlotDigitizer software to extract quantitative data from the figure. Results were expressed as standardized mean difference (SMD) and 95% confidence intervals (CI) when the outcome is measured in different ways; otherwise, the mean difference (MD) and 95% CI were calculated. When there was a sufficient number of studies, subgroup analysis was performed on muscle type, atrophy model, atrophy duration, and hypertrophy percentage in the animal

Figure 1 PRISMA flow diagram of study selection.
| Author et al. (year) | Participants (number, sex) | Age | Muscle | Hypertrophy/Atrophy model | Training/Atrophy duration |
|----------------------|-----------------------------|-----|--------|---------------------------|--------------------------|
| Kadi et al. (2004)   | Young (15, M)               | 24 ± 1| VL     | Resistance training       | 12 wk                    |
| Psilander et al. (2019) | Young (10, W & 9, M)     | 25 ± 1| VL     | Resistance training       | 10 wk                    |
| Snijders et al. (2019) | Old (53, M/W)              | 70 ± 6| VL     | Resistance training       | 24 wk                    |
| Carlson et al. (2009) | Young (11, M); Old (9,M)   | 22 ± 2| VL     | Leg immobilization        | 12 wk                    |
| Dirk et al. (2014)   | Old (12, M)                 | 69 ± 1| VL     | Leg immobilization        | 2 wk                     |
| Snijders et al. (2014) | Young (12, M)              | 24 ± 1| VL     | Leg immobilization        | 2 wk                     |
| Dirk et al. (2014b)  | Young (12, M)               | 23 ± 1| VL     | Leg immobilization        | 5 d                      |
| Sueta et al. (2013)  | Young (11, M); Old (9,M)   | 25 ± 4| VL     | Leg immobilization        | 2 wk                     |
| Ohira et al. (1999)  | Young (13, M)               | 33 ± 3| VL     | Bed rest                 | 2 and 4 mos              |
| Brooks et al. (2010) | Young (7, M)                | 40 ± 15| VL    | Bed rest                 | 28 d                     |
| Arentson-Lantz et al. (2016) | Young (7, M/W)   | 51 ± 1| VL     | Bed rest                 | 2 wk                     |
| Reider et al. (2017) | Old (9, M/W)                | 69 ± 2| VL     | Bed rest                 | 5 d                      |
| Reider et al. (2018) | Young (14, M/W); Old (9, M/W) | 23 ± 1; 66 ± 1 | VL  | Bed rest                 | 5 d                      |
| Moore et al. (2018)  | Old (14, M)                 | 71 ± 5| VL     | Step reduction            | 14 d                     |
| Reider et al. (2019) | Old (12, M)                 | 70 ± 2| VL     | Step reduction            | 7 and 14 d               |
| Smith et al. (2013)  | Young (8, M/W); CP (8, M/W) | 16 ± 2; 11 ± 4 | VL  | CP                       | NA                       |
| Davaneti et al. (2016) | Children (6, M)           | 13 ± 3| VL     | CP                       | NA                       |
| Von Walden et al. (2018) | Children and adolescents (22, M/W) | 15 ± 7| VL    | CP and brain injury      | NR                       |
| Eliason et al. (2009) | Old (12, M/W); Moderate COPD (12, M/W); Severe COPD (11, M/W) | 62 ± 6.6| Tibial anterior COPD | NR                       |
| Menon et al. (2012)  | Old (7, M/W); COPD (12, M/W); Severe COPD (11, M/W) | 67 ± 2| VL    | COPD                     | NR                       |
| Thériault et al. (2012) | Old (12, M/W); Moderate COPD (12, M/W); Severe COPD (11, M/W) | 67 ± 3; 64 ± 2| VL  | COPD                     | NR                       |
| Sancho-Muñoz et al. (2021) | Old (13, M/W); Non SAR (19, M/W); SAR (26, M/W) | 66 ± 5; 65 ± 7| VL  | COPD                     | NR                       |
| Noehren et al. (2015) | Young (10, M/W)            | 23 ± 5| VL     | ACL injury               | 12 wk                    |
| Fry et al. (2017)    | Young (10, M/W)             | 23 ± 5| VL     | ACL injury               | 8 wk                     |
| Parstoever et al. (2021) | Young (1, W; 15, M)       | 26 ± 4| VL     | ACL injury               | 12 wk                    |
| Day et al. (1995)    | Young (5, M/W)              | 40 ± 7| VL     | Space flight             | 11 d                     |
| Dirk et al. (2015)   | Old (6, M/W)                | 63 ± 6| VL     | ICU patients             | NA                       |
| Kramer et al. (2017) | Old (30, F)                 | 80 ± 2| VL     | Hip fracture             | NR                       |
| Farup et al. (2016)  | Young (32, NR)              | 46 ± 1| VL     | Multiple sclerosis       | NR                       |
| Shao et al. (2020)   | Young (12, M/W)             | 14 ± 4| VL     | Idiopathic scoliosis     | NR                       |
| Verdijk et al. (2012) | Young (8, M)               | 31 ± 3| VL     | Spinal cord injury       | 9 years                  |
| D’Souza et al. (2016) | Young (11, M)              | 20 ± 2| VL     | Type 1 diabetes          | NR                       |
| Author                      | Detraining duration | Muscle fibre size            | Myonuclear content | Myonuclear domain | SC content |
|----------------------------|---------------------|------------------------------|--------------------|-------------------|------------|
| Kadi et al. (2004)         | 12 wk               | Training: Mixed: ↑ Detraining: Mixed: ↓ | Training: Mixed: ↓ | Detraining: Mixed: ↓ | NM         |
| Psilander et al. (2019)    | 20 wk               | Training: Mixed, I, II: ↔ | Training: Mixed, I, II: ↔ | Detraining: Mixed, I, II: ↔ | NM         |
| Snijders et al. (2019)     | 48 wk               | Training: Mixed, I, II: ↔ | Training: Mixed, I, II: ↔ | Detraining: Mixed, I, II: ↔ | NM         |
| Blocquiaux et al. (2020)   | 12 wk               | Training: Mixed, I, II: ↔ | Training: Mixed, I, II: ↔ | Detraining: Mixed, I, II: ↔ | NM         |
| Carlson et al. (2009)      | NA                  | Young and Old: Mixed: ↓    | Mixed: ↓           | Mixed: ↓          | NM         |
| Dirks et al. (2014a)       | NA                  | Mixed: ↓                   | Mixed: ↓           | Mixed: ↓          | NM         |
| Snijders et al. (2014)     | NA                  | Young: I, II: ↔ Old: I, II: ↔ | Mixed: 2 mon: ↔, 4 mon: ↓ | Mixed: 2 and 4 mos: ↔ | NM         |
| Suettet al. (2013)         | NA                  | Mixed: ↓                   | Mixed: I, II: ↔    | Mixed: I, II: ↔   | Mixed: 2 and 4 mos: ↔ | Mixed: 2 mos: ↔, 4 mos: ↓ | NM         |
| Ohira et al. (1999)        | NA                  | Mixed: ↓                   | Mixed: I, II: ↓    | Mixed: I, II: ↓   | Mixed: ↓ |
| Brooks et al. (2010)       | NA                  | Mixed: ↓                   | Mixed: I, II: ↓    | Mixed: I, II: ↓   | Mixed: ↓ |
| Arentson-Lantz et al. (2016)| NA          | Mixed: I, II: ↓            | Mixed: I, II: ↓    | Mixed: I, II: ↓   | Mixed: ↓ |
| Von Walden et al. (2018)   | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Eliason et al. (2009)      | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Menon et al. (2012)        | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Thériault et al. (2012)    | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Sancho-Muñoz et al. (2021) | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Noeheren et al. (2016)     | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Fry et al. (2017)          | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Parstorfer et al. (2021)   | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Day et al. (1995)          | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Dirks et al. (2015)        | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Kramer et al. (2017)       | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Farup et al. (2016)        | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Shao et al. (2020)         | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| Verdijk et al. (2012)      | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |
| D’Souza et al. (2016)      | NA                  | Mixed: ↑                   | Mixed: ↑           | Mixed: ↑          | Mixed: ↓ |

↑, significantly higher compared with control values; ↓, significantly lower compared with control values; ↔, no difference between experiment and control values; ACL, anterior cruciate ligament; COPD, chronic obstructive pulmonary patients; CP, cerebral palsy; I, Type I muscle fibres; II, Type II muscle fibres; M, men; M/W, men and women combined; Mixed, mixed muscle fibre type; NA, not applicable; NM, not measured; NR, not reported; SAR, sarcopenic patients; VL, vastus lateralis; W, women.

This study is performed in both muscle cross section and single muscle fibre. This study is performed in single muscle fibre.
| Author                  | Age, years (number) | Gender | Muscle | Muscle fibre size | Myonuclear content | Myonuclear domain | SC number |
|-------------------------|---------------------|--------|--------|-------------------|--------------------|-------------------|-----------|
| Vassilopoulos et al. (1977) | 12–30 (6) vs. 60–71 (6) | M/W Vl | Mixed: ↔ | Mixed: ↔ | NM | NM |
| Manta et al. (1987) | 17–30 (4) vs. > 60 (7) | M/W Vl | Mixed: ↓ | Mixed: ↔ | NM | ↑ NM |
| Hikida et al. (1989) | 17–26 (7) vs. 59–71 (8) | M | Mixed, I, II: ↓ | Mixed: ↔ | NM | ↑ NM |
| Roth et al. (2000) | 22–28 (7) vs. 66–72 (8) | M/W Vl | NM | NM | Mixed: ↔ | ↑ NM |
| 25–27 (7) vs. 64–71 (7) | W | | | | Mixed: ↔ | ↓ NM |
| Renaut et al. (2002) | 22–24 (6) vs. 70–78 (6) | M/W Biceps | NM | Mixed: ↔ | NM | ↓ NM |
| Sajko et al. (2002) | 24–38 (4) vs. 67–73 (6) | M | VL | NM | Mixed: ↓ | ↑ NM |
| Renault et al. | 23–29 (15) vs. 70–78 (13) | M | VL | NM | Mixed: ↑ | ↓ NM |
| 20–26 (16) vs. 73–79 (14) | W | | Mixed: ↑ | | Mixed: ↓ | ↓ NM |
| Sajko et al. (2004) | 26–30 (6) vs. 69–71 (6) | M | VL | NM | NM | NM |
| Dreyer et al. (2006) | 21–35 (10) vs. > 60 (9) | M | VL | I: ↔, II: ↓ | Mixed: ↔ | ↑ NM |
| Petrella et al. (2006) | 20–35 (15) vs. 60–75 (13) | M | VL | Mixed: ↔ | Mixed: ↔ | ↓ NM |
| 20–35 (16) vs. 60–75 (14) | W | | Mixed: ↔ | | Mixed: ↓ | ↓ NM |
| Mohamed et al. (2007) | 24–50 (7) vs. 65–81 (9) | NR Triceps | Mixed: ↓ | NM | NM | ↓ NM |
| Verdijk et al. (2007) | 19–21 (8) vs. 69–71 (8) | M | VL | I: ↔, II: ↓ | I: I; I: ↑; II: ↔ | ↑ NM |
| Cristea et al. (2010) | 21–32 (6) vs. 72–96 (9) | M | VL | I: ↑, II: ↓ | I: ↑, II: ↔ | ↑ NM |
| McKay et al. (2012) | 24–32 (6) vs. 65–96 (9) | W | | | | ↑ NM |
| Verdijk et al. (2012) | 28–34 (8) vs. 73–77 (8) | M | VL | I: ↔, II: ↓ | I: ↔, II: ↔ | ↑ NM |
| Walker et al. (2012) | 25–29 (5) vs. 68–72 (6) | W | VL | I: ↔, II: ↓ | Mixed: ↓ | ↓ NM |
| 25–29 (5) vs. 68–72 (6) | W | | Mixed: ↔ | | Mixed: ↓ | ↓ NM |
| Suett et al. (2013) | 21–30 (11) vs. 61–74 (9) | M | VL | I: I; II: ↔ | NM | ↑ NM |
| McKay et al. (2014) | 21–27 (12) vs. 62–70 (12) | M | VL | I: I; II: ↔ | NM | ↑ NM |
| Sniijders et al. (2014) | 21–23 (10) vs. 72–74 (10) | M | VL | I: ↔, II: ↓ | NM | ↑ NM |
| Verdijk et al. (2014) | 18–49 (50) vs. > 70 (49) | M | VL | I: ↔, II: ↓ | I: ↔, II: ↓ | ↑ NM |
| Verdijk et al. (2016) | 24–28 (14) vs. 71–73 (16) | M | VL | I: ↔, II: ↓ | I: ↔, II: ↓ | ↑ NM |
| 21–24 (23) vs. 63–71 (22) | M | VL | I: ↔, II: ↓ | I: ↔, II: ↓ | ↑ NM |
| Kramer et al. (2017) | 18–25 (15) vs. > 65 (15) | W | VL | I: ↔, II: ↓ | I: ↔, II: ↓ | ↑ NM |
| Kelly et al. (2018) | 22–30 (27) vs. 62–70 (91) | M | VL | I: ↔, II: ↓ | I: ↔, II: ↓ | ↑ NM |
| Reidt et al. (2018) | 18–35 (14) vs. 60–75 (9) | M/W Vl | Mixed: ↔ | Mixed: ↔ | NM | NM |
| Karlsson et al. (2019) | 19–23 (9) vs. 70–84 (18) | M/W Vl | I: ↔, II: ↓ | I: ↔, II: ↓ | ↑ NM |
| Naro et al. (2019) | 22–28 (6) vs. 81–96 (6) | M/W Vl | I: ↔, II: ↓ | I: ↔, II: ↓ | ↑ NM |
| Karlsson et al. (2020) | 22–28 (7) vs. 63–71 (19) | M | VL | I: ↔, II: ↓ | NM | ↑ NM |
| Perez et al. (2021) | 20–24 (6) vs. 65–78 (11) | M/W Vl | NM | NM | Mixed: ↓ | ↓ NM |

↑: significantly higher compared with control values; ↓: significantly lower compared with control values; ↔, no difference between experiment and control values; I, I: Type I muscle fibres; II, II: Type II muscle fibres; M, men; M/W, men and women combined; Mixed, mixed muscle fibre type; NM, not measured; VL, vastus lateralis; W, women.

This study is performed in single muscle fibre.

*This study is performed in both muscle cross-section and single muscle fibre.
| Author                     | Specie (sex)          | Muscle     | Hypertrophy/atrophy model          | Training/atrophy duration |
|----------------------------|-----------------------|------------|------------------------------------|---------------------------|
| Bruusgaard et al. (2010)   | NMRI mice (F)         | EDL        | Synergist ablation                 | 14 d                      |
| Lee et al. (2018)          |                      |            |                                    |                           |
| Dungan et al. (2019)       | Sprague–Dawley rats (F) | FHL        | Weight loaded-ladder climbing      | 8 wk                      |
| Murach et al. (2020)       | CS7BL/6J mice (F)     | Plan       | Weighted wheel running             | 8 wk                      |
| Eftestøl et al. (2021)     | Sprague–Dawley rats (M) | Sol, Gas, Plan | Weighted wheel running             | 8 wk                      |
| Hyatt et al. (2003)        | Sprague–Dawley rats (F) | Sol        | Climbing                           | 5 wk                      |
| Kasper et al. (1996a)      | Sprague–Dawley rats (F) | Gas, TA    | Suspension                         | 5.4 d                     |
| Bruusgaard et al. (2008)   | NMRI mice (F)         | EDL, Sol   | Suspension                          | 3, 7, 14, and 21 d        |
| Ontell (1974)              |                      |            |                                    |                           |
| Cardasis & Cooper (1975)   |                      |            |                                    |                           |
| Snow (1983)                |                      |            |                                    |                           |
| Maltin et al. (1992)       |                      |            |                                    |                           |
| Irintchev et al. (1994)    |                      |            |                                    |                           |
| Allen et al. (1995)        |                      |            |                                    |                           |
| Vigue et al. (1997)        |                      |            |                                    |                           |
| Dupont-Versteegden et al. (1999) |            |            |                                    |                           |
| Milanic et al. (1999)      |                      |            |                                    |                           |
| Schmalbruch et al. (2000)  |                      |            |                                    |                           |
| Dedkov et al. (2001)       |                      |            |                                    |                           |
| Nnorim (2001)              |                      |            |                                    |                           |
| Wada et al. (2002)         |                      |            |                                    |                           |
| Dedkov et al. (2003)       |                      |            |                                    |                           |
| Roy et al. (2005)          |                      |            |                                    |                           |
| Zhong et al. (2005)        |                      |            |                                    |                           |
| Araavamudan et al. (2006)  |                      |            |                                    |                           |
| Van Der Merr et al. (2011) |                      |            |                                    |                           |
| Liu et al. (2015)          |                      |            |                                    |                           |
| Aguera et al. (2019)       |                      |            |                                    |                           |
| Choi et al. (2020)         |                      |            |                                    |                           |
| Hansson et al. (2020)      |                      |            |                                    |                           |
| Xing et al. (2020)         |                      |            |                                    |                           |
| Wong et al. (2021)         |                      |            |                                    |                           |
| Darr et al. (1989)         |                      |            |                                    |                           |
| Kasper et al. (1996b)      |                      |            |                                    |                           |
| Allen et al. (1997)        |                      |            |                                    |                           |
| Mozdziak et al. (2000)     |                      |            |                                    |                           |
| Mitchell et al. (2001)     |                      |            |                                    |                           |
| Yamazaki (2003)            |                      |            |                                    |                           |
| Mitchell and Pavlath (2004) |                      |            |                                    |                           |
| Leeuwenburgh et al. (2005) |                      |            |                                    |                           |
| Ferreira et al. (2006)     |                      |            |                                    |                           |
| Wang et al. (2006)         |                      |            |                                    |                           |
| Kawano et al. (2007)       |                      |            |                                    |                           |
| Kawano et al. (2008)       |                      |            |                                    |                           |
| Oishi et al. (2008)        |                      |            |                                    |                           |

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| Author                  | Specie (sex)                      | Muscle | Hypertrophy/atrophy model | Training/atrophy duration |
|-------------------------|-----------------------------------|--------|---------------------------|--------------------------|
| Tarakina et al. (2008)  | Wistar rats (M)                   | Sol    | Suspension                | 14 d                     |
| Matsuba et al. (2009)   | CS7BL6 mice (M)                   | Sol    | Suspension                | 14, 28, and 42 d         |
| Kartschikina et al. (2010) | Wistar rats (M)               | Sol    | Suspension                | 14 d                     |
| Zhang et al. (2010)     | Wistar rats (M)                   | EDL, Sol | Suspension             | 28 d                     |
| Kachaeva et al. (2011)  | Wistar rats (M)                   | Sol    | Suspension                | 14 d                     |
| Ohira et al. (2011)     | Wistar rats (M)                   | Adductor longus | Suspension              | 16 and 32 d             |
| Teixeira et al. (2011)  | Charles River mice (M)            | Sol    | Suspension                | 1, 2, 3, and 8 d        |
| Brunsgaard et al. (2012) | PAX7-DTA mice (F)              | Sol    | Suspension                | 2, 4, and 14 d          |
| Guo et al. (2012)       | BALB/c mice (M)                   | Sol    | Suspension                | 14 d                     |
| Ohira et al. (2012)     | Wistar rats (M)                   | Sol    | Suspension                | 14 d                     |
| Itoh et al. (2012)      | ICR mice (M)                      | Sol    | Suspension                | 14 d                     |
| Anderson et al. (2018)  | CS7BL6 mice (M/F)                 | Sol    | Suspension                | 18 d                     |
| Brooks et al. (2018)    | CS7BL6 mice (M/F)                 | Sol    | Suspension                | 14 d                     |
| Miller et al. (2018)    | Norway-F344 rats (M)              | Gas    | Suspension                | 14 d                     |
| Kneppers et al. (2019)  | CS7BL6 mice (M)                   | Gas    | Suspension                | 14 d                     |
| Nakashishi et al. (2021) | Wistar rats (F)                | Sol    | Suspension                | 14 d                     |
| Petrocelli et al. (2021) | C57BL6 mice (M)               | Gas, Sol | Suspension            | 14 d                     |
| Smith et al. (2000)     | Californian rabbits (F)           | Sol    | Immobilization            | 2 and 6 d                |
| Wanek and Snow (2000)   | Sprague-Dawley rats (M/F)         | Sol    | Immobilization            | 2, 4, and 8–10 wk       |
| Ye et al. (2013)        | CS7BL6 mice (F)                   | Sol    | Immobilization            | 14 d                     |
| Matsumoto et al. (2014) | 5| Wistar rats (M)                   | Gas    | Immobilization            | 4 wk                     |
| Li et al. (2016)        | 5| Wistar rats (M)                   | Sol    | Immobilization            | 14 d                     |
| Guitart et al. (2018)   | 5| CS7BL6 mice (F)                   | Gas, Sol | Immobilization        | 7 d                      |
| Usuki et al. (2019)     | 5| Wistar rats (M)                   | Sol    | Immobilization            | 7 d                      |
| Suzuki et al. (2020)    | 5| Sprague-Dawley rats (M)           | Plan, Sol | Immobilization    | 7 d                      |
| Zazu et al. (2020)      | 5| Wistar rats (M)                   | TA     | Immobilization            | 7 d                      |
| Honda et al. (2021)     | 5| Wistar rats (M)                   | Sol    | Immobilization            | 14 d                     |
| Allen et al. (1996)     | 5| Sprague-Dawley rats (M)           | Sol    | Space flight              | 14 d                     |
| Hikida et al. (1997)    | 5| Fisher 344 rats (M)               | Sol    | Space flight              | 10 d                     |
| Sandona et al. (2012)   | 5| CS7BL101 mice (M)                 | EDL, Sol | Space flight            | 91 d                     |
| Raduina et al. (2018)   | 5| CS7BL6 mice (M)                   | Quadriceps | Space flight        | 30 d                     |
| McClung et al. (2006)   | 5| Sprague-Dawley rats (F)           | Diaphragm | Mechanical ventilation | 12 h                     |

1, significantly higher compared with control values; ↓, significantly lower compared with control values; ↔, no difference between experiment and control values; EDL, extensor digitorum longus; F, female; FHL, flexor hallucis longus; Gas, gastrocnemius muscle; ICR, Institute of Cancer Research (ICR) mice (Japan SLC, Shizuoka, Japan); M, male; M/F, male and female combined; MG, medial gastrocnemius; NA, not applicable; NM, not measured; Plan, plantaris muscle; Sol, soleus muscle; TA, tibialis anterior muscle.

This study is performed in single muscle fibre.
| Author                        | Detraining duration | Myonuclear content | Satellite cell number |
|------------------------------|---------------------|--------------------|-----------------------|
| Bruusgaard et al. (2010) a16 | 2/8 wk denervation  | In vivo Training: ⇧, Detraining: ↔ | NM                    |
|                              |                     | Ex vivo Training: ⇧, Detraining: ↔ |                      |
| Lee et al. (2018) a18        | 20 wk               |                    | NM                    |
| Dungan et al. (2019) b21     | 12 wk               |                    | Muscle cross section  |
|                              |                     | Training: ↑, Detraining: ↓ |                      |
| Murach et al. (2020) b22     | 24 wk               |                    | NM                    |
| Eftestøl et al. (2021) 92    | 10 wk               |                    | NM                    |
| Hyatt et al. (2003) 51       | NA                  |                    | NM                    |
| Kasper et al. (1996a) 569    | NA                  |                    | NM                    |
| Bruusgaard et al. (2008) 917 | NA                  |                    | NM                    |
| Ontell (1974) 552            | NA                  |                    | NM                    |
| Cardasis & Cooper (1975) 553 | NA                  |                    | NM                    |
| Snow (1983) 54               | NA                  |                    | NM                    |
| Maltin et al. (1992) 55      | NA                  |                    | NM                    |
| Irintchev et al. (1994) 56   | NA                  |                    | NM                    |
| Allen et al. (1995) 557      | NA                  |                    | NM                    |
| Vigue et al. (1997) 558      | NA                  |                    | NM                    |
| Dupont-Versteegden et al. (1999) 25 | NA | | NM                  |
| Milanic et al. (1999) 589    | NA                  |                    | NM                    |
| Dupont-Versteegden et al. (2000) 510 | NA | | NM                    |
| Schmalbruch et al. (2000) 511 | NA                  |                    | NM                    |
| Dedkov et al. (2001) 512     | NA                  |                    | NM                    |
| Nnodim (2001) 513            | NA                  |                    | NM                    |
| Wada et al. (2002) 514       | NA                  |                    | NM                    |

Denervation
3 d in MG, TA: ↔
14, 28 d in MG, TA: ↑
Spinal cord transection
3, 14, 28 d in MG: ↔
3, 28 d in TA: ↔
14 d in TA: ↑
NM

Muscle cross-section
Detraining: 3, 14, and 21 d in EDL: ↔
TTX blockade: 14 and 21 d in EDL: ↔
Muscle cross-section
Detraining: 3, 7, 14, and 21 d in EDL& Sol: ↔
2 and 3 wk: ↑
1, 2, 3, 7, 14, 18, and 28 d: ↔
2 and 3 wk: ↔
3, 7, 14, 23, and 65 d in EDL & Sol: ↔
30d in EDL: ↑, 30 and 45 d in Sol: ↑
5 and 7 d: ↑
2 mos: ↑
4 mos: ↔
7 mos: ↓
NM

MTX blockade
↓↓
NM

Endotoxin
↓↓
NM

3 weeks old (5, 10 d): ↓
4 months old (10, 120 d): ↔
NM
| Author                          | Detraining duration | Myonuclear content         | Satellite cell number |
|--------------------------------|---------------------|-----------------------------|-----------------------|
| Dedkov et al. (2003)\textsuperscript{1}\textsuperscript{5} | NA                  | NM                          |                       |
| Roy et al. (2005)\textsuperscript{6} | NA                  | 4 d in MG: ↔                |                       |
|                                |                     | 60 d in MG: ↓               |                       |
|                                |                     | 4 d and 60 d in TA: ↔       |                       |
| Zhong et al. (2005)\textsuperscript{7} | NA                  | 4 d: ↔                      |                       |
|                                |                     | 60 d: ↔                     |                       |
| Aravamudan et al. (2006)\textsuperscript{8}\textsuperscript{18} | NA                  | ↔                           |                       |
|                                |                     | 5-month-age rats             |                       |
|                                |                     | 1, 2, 4 wk: ↔               |                       |
|                                |                     | 25-month-age rats            |                       |
|                                |                     | 1, 2, and 4 wk: ↔           |                       |
| Van Der Merr et al. (2011)\textsuperscript{9} | NA                  | 1, 2, 4 wk: ↔               |                       |
|                                |                     | 25-month-age rats            |                       |
|                                |                     | 1 wk: ↑, 2 and 4 wk: ↔      |                       |
|                                |                     | ↑                            |                       |
|                                |                     | ↓                            |                       |
| Liu et al. (2015)\textsuperscript{10} | NA                  | 2, 4, and 6 wk: ↓           |                       |
|                                |                     | NM                          |                       |
| Aguera et al. (2019)\textsuperscript{11} | NA                  | NM                          |                       |
| Choi et al. (2020)\textsuperscript{12} | NA                  | ↔                           |                       |
| Hansson et al. (2020)\textsuperscript{13} | NA                  | ↔                           |                       |
| Xing et al. (2020)\textsuperscript{14} | NA                  | ↔                           |                       |
| Wong et al. (2021)\textsuperscript{15} | NA                  | ↔                           |                       |
| Darr et al. (1989)\textsuperscript{16} | NA                  | Single muscle fibre          |                       |
|                                |                     | 3 d in Sol: ↔               |                       |
|                                |                     | 10, 20, 30 d in Sol: ↓      |                       |
|                                |                     | 3, 10, and 30 d in EDL: ↔   |                       |
|                                |                     | 20 d in EDL: ↓              |                       |
| Kasper et al. (1996)\textsuperscript{17} | NA                  | Muscle cross-section         |                       |
|                                |                     | 3 d in Sol: ↔               |                       |
|                                |                     | 10, 20, 30 d in Sol: ↓      |                       |
|                                |                     | 3, 10, 20, and 30 d in EDL: ↔ |                       |
|                                |                     | Sol: ↑, Plan: ↔            |                       |
| Allen et al. (1997)\textsuperscript{18} | NA                  | ↓                           |                       |
| Mozdziak et al. (2000)\textsuperscript{19} | NA                  | ↓                           |                       |
| Mitchell et al. (2001)\textsuperscript{20} | NA                  | ↓                           |                       |
| Yamazaki (2003)\textsuperscript{21} | NA                  | ↓                           |                       |
| Mitchell and Pavlath (2004)\textsuperscript{22} | NA                  | ↓                           |                       |
| Leeuwenburgh et al. (2005)\textsuperscript{23} | NA                  | ↓                           |                       |
| Ferreira et al. (2006)\textsuperscript{24} | NA                  | 6 mos: ↓, 32 mos: ↔        |                       |
| Wang et al. (2006)\textsuperscript{25} | NA                  | ↓                           |                       |
| Kawanoto et al. (2007)\textsuperscript{26} | NA                  | ↓                           |                       |
| Kawanoto et al. (2008)\textsuperscript{27} | NA                  | ↓                           |                       |
| Oishi et al. (2008)\textsuperscript{28} | NA                  | ↓                           |                       |
| Tarakina et al. (2008)\textsuperscript{29} | NA                  | ↓                           |                       |
| Matsuba et al. (2009)\textsuperscript{30} | NA                  | ↓                           |                       |
| Kartashkina et al. (2010)\textsuperscript{31} | NA                  | ↓                           |                       |
| Zhang et al. (2010)\textsuperscript{32} | NA                  | ↓                           |                       |
| Kachaeva et al. (2011)\textsuperscript{33} | NA                  | ↓                           |                       |
| Ohira et al. (2011)\textsuperscript{34} | NA                  | ↓                           |                       |
| Teixeira et al. (2011)\textsuperscript{35} | NA                  | ↓                           |                       |
| Brunsgaard et al. (2012)\textsuperscript{36} | NA                  | ↓                           |                       |
| Jackson et al. (2012)\textsuperscript{37} | NA                  | ↓                           |                       |
| Guo et al. (2012)\textsuperscript{38} | NA                  | ↓                           |                       |
| Lomonosova et al. (2012)\textsuperscript{39} | NA                  | ↓                           |                       |
Table 3 (continued)

| Author                          | Detraining duration | Myonuclear content | Satellite cell number |
|---------------------------------|---------------------|--------------------|-----------------------|
| Zushi et al. (2012)             | NA                  | ↓                  | NM                    |
| Itoh et al. (2014)              | NA                  | ↓                  | NM                    |
| Park et al. (2014)              | NA                  | NM                 | ←                    |
| Babcock et al. (2015)           | NA                  | ↓                  | ↓                     |
| Ohira et al. (2015)             | NA                  | In +/+ , +/op and op/op: ↓ | In +/+ , +/op and op/op: ↓ |
| Nakanish et al. (2016)          | NA                  | ↓                  | ↓                     |
| Itoh et al. (2017)              | NA                  | ↔                  | NM                    |
| Anderson et al. (2018)          | NA                  | NM                 | ↓                     |
| Brooks et al. (2018)            | NA                  | ↓                  | ↔                    |
| Miller et al. (2018)            | NA                  | ↔                  | ↓                     |
| Kneppers et al. (2019)          | NA                  | ↓                  | ↔                    |
| Nakanishi et al. (2021)         | NA                  | NM                 | ↓                     |
| Petrocelli et al. (2021)        | NA                  | NM                 | Gas, Sol: ↔          |
| Smith et al. (2000)             | NA                  | 2 d: ↔, 6 d: ↓   | NM                    |
| Wanek and Snow (2000)           | NA                  | NM                 | 2 and 4 wk: ↔, 8–10 wk: ↓ |
| Ye et al. (2013)                | NA                  | NM                 | ↓                     |
| Matsumoto et al. (2014)         | NA                  | ↓                  | NM                    |
| Li et al. (2016)                | NA                  | NM                 | ↓                     |
| Guitart et al. (2018)           | NA                  | NM                 | Gas: ↓, Sol: ↓       |
| Usuki et al. (2019)             | NA                  | NM                 | ↓                     |
| Suzuki et al. (2020)            | NA                  | Plan, Sol: ↔      | NM                    |
| Zazuła et al. (2020)            | NA                  | ↓                  | NM                    |
| Honda et al. (2021)             | NA                  | ↓                  | NM                    |
| Allen et al. (1996)             | NA                  | ↓                  | NM                    |
| Hikida et al. (1997)            | NA                  | ↓                  | NM                    |
| Sanonâ et al. (2012)            | NA                  | EDL: ↔, Sol: ↓    | NM                    |
| Radugina et al. (2018)          | NA                  | ↓                  | NM                    |
| McClung et al. (2006)           | NA                  | ↓                  | NM                    |

↑, significantly higher compared with control values; ↓, significantly lower compared with control values; ↔, no difference between experiment and control values; EDL, extensor digitorum longus; F, female; FHL, flexor hallucis longus; Gas, gastrocnemius muscle; ICR, Institute of Cancer Research (ICR) mice (Japan SLC, Shizuoka, Japan); M, male; M/F, male and female combined; MG, medial gastrocnemius; NA, not applicable; NM, not measured; Plan, plantaris muscle; Sol, soleus muscle; TA, tibialis anterior muscle.

This study is performed in single muscle fibre.

This study is performed in both muscle cross-section and single muscle fibre.
model and on age (young and old) and atrophy model in human subjects. To evaluate and ensure the robustness of the results, sensitivity analysis was carried out by removing studies from the meta-analysis. Sensitivity analysis showed that no results were affected by any study (data not shown). Finally, funnel plots with Egger weighted regression test were used for assessing publication bias using STATA version 16.

**Results**

**Evidence from human studies**

**Skeletal muscle responses to hypertrophy**

Four reports involving 117 participants assessed the response of skeletal muscle (vastus lateralis) to resistance training followed by a period of detraining.\(^{29,30,35,36}\) Resistance training duration ranged from 10 to 24 weeks in these studies. However, detraining duration ranged from 12 to 48 weeks. Currently, the general consensus is that myonuclear content tends to be lower in older adults (≥60 year) compared with young adults (18–55 year).\(^{37}\) Thus, we performed a subgroup analysis to clarify the effects of an episode of overload hypertrophy and subsequent disuse atrophy on the present review outcomes in terms of the different age categories. The details of the included studies are shown in Table 1.

**Myofibre size following training and detraining** Resistance training significantly increased cross-sectional area (CSA) compared with baseline values (mean: MD = 650.32, 95% CI 355.30–945.34, \(P = 0.0001\); Type I: MD = 470.83, 95% CI 168.29–773.37, \(P = 0.002\); Type II: MD = 723.93, 95% CI 358.02–1089.84, \(P = 0.0001\); Figure S2A–S2C). Further, CSA after a detraining period following resistance training returned to the pre-training values (mean: MD = 83.46, 95% CI –649.41 to 816.32, \(P = 0.82\); Type I: MD = 104.39, 95% CI –604.64 to 813.23, \(P = 0.77\); Type II: MD = 190.74, 95% CI –882.92 to 1264.40, \(P = 0.73\); Figure S2D–S2F). Subgroup analysis in mixed and Type II fibres showed no statistically significant difference between young and old adults after training and detraining periods (mixed: \(P = 0.50\) and \(P = 0.20\); Type II: \(P = 0.97\) and \(P = 0.31\), respectively). Further, subgroup analysis showed that the reduction of Type I fibre CSA of young adults was significantly higher following a detraining period than old subjects (\(P = 0.03\)) (Figure S2A–S2F).

**Myonuclear content following training and detraining** Resistance training significantly increased myonuclear content in mixed and Type II fibres compared with baseline values (mean: MD = 0.12, 95% CI 0.00–0.23, \(P = 0.04\); Type I: MD = 0.04, 95% CI –0.08 to 0.15, \(P = 0.55\); Type II: MD = 0.23, 95% CI 0.07–0.40, \(P = 0.006\); Figure S2G–S2I). Compared with pre-training, there was a significant difference in myonuclear content after a detraining period (mean: MD = –0.14, 95% CI –0.26 to –0.02, \(P = 0.02\); Type I: MD = –0.14, 95% CI –0.28 to –0.0, \(P = 0.05\); Type II: MD = –0.23, 95% CI –0.37 to –0.10, \(P = 0.0009\); Figure S2J–S2L), indicating that myonuclear content after a detraining period was less than the baseline. Subgroup analysis showed no statistically significant difference between young and old adults after training and detraining periods (mixed: \(P = 0.56\) and \(P = 0.73\); Type I: \(P = 0.42\) and \(P = 0.86\); Type II: \(P = 0.37\) and \(P = 0.73\), respectively; Figure S2G–S2L).

**Myonuclear domain following training and detraining** A single report with 19 participants assessed myonuclear content in single muscle fibre using 44–57 fibres from each biopsy sample.\(^{29}\) This study reported no change in myonuclear content in response to resistance training (i.e. +5%) and after detraining (i.e. +3%).

**Satellite cell number following training and detraining**

Three studies involving 94 participants assessed SC abundance.\(^{30,35,36}\) Resistance training significantly increased SC abundance in mixed and Type I fibres compared to baseline values (mean: SMD = 0.75, 95% CI 0.33–1.18, \(P = 0.0005\); Type I: SMD = 0.36, 95% CI –0.14 to 0.85, \(P = 0.16\); Type II: SMD = 0.81, 95% CI 0.30–1.32, \(P = 0.002\); Figure S2S–S2T). Additionally, SC abundance after a detraining period returned to pre-training levels (mean: SMD = 0.16, 95% CI –0.32 to 0.64, \(P = 0.52\); Type I: SMD = –0.01, 95% CI –0.66 to 0.65, \(P = 0.99\); Type II: SMD = 0.09, 95% CI –0.57 to 0.74, \(P = 0.79\); Figure S2W–S2Y). Subgroup analysis in mixed fibres showed no statistically significant difference between young and old adults after training and detraining periods (\(P = 0.29\) and \(P = 0.58\), respectively). The number of studies was too small to permit subgroup analyses of Type I or Type II fibres (Figure S2I–S2Y).

**Skeletal muscle responses to atrophy**

Twenty-nine studies assessed skeletal muscle growth in whole muscle cross section in response to leg
immobilization, bed rest, step reduction, space flight, and patients suffering from cerebral palsy, chronic obstructive pulmonary disease (COPD), anterior ligament reconstruction, fully sedating ICU patients, hip fracture, multiple sclerosis, adolescent idiopathic scoliosis, spinal cord injury, and Type1 diabetes.

The details of the included studies are shown in Table 1. We performed subgroup analyses to determine the potential impact that differences in the age of the participants (old vs. young), the duration of the intervention (≤5 days, 7–14 days, 20–30 days, and ≥60 days), and the model of atrophy used had on the atrophic response.

**Myofibre size following atrophy**
Analysis of 19 studies involving 460 participants found there was lower skeletal muscle CSA following the aforementioned ‘Skeletal muscle responses to atrophy’ section interventions (mixed: MD = −497.24, 95% CI −734.13 to −260.35, P = 0.0001; Type I: MD = −743.63, 95% CI −1059.28 to −427.98, P = 0.0001; Type II: MD = −908.11, 95% CI −1268.67 to −547.54, P = 0.0001; Figure S3A–S3C). Subgroup analysis showed no statistical significant difference between young and old adults for CSA of mixed, Type I, and Type II fibres in response to atrophy (P = 0.52, P = 0.93, and P = 0.60, respectively). Stratifying studies based on the duration of the intervention period found that myofibre CSA was decreased after 7 days in different atrophy models (mixed: MD = −914.33, 95% CI −1528.91 to −299.75, P = 0.004; Type I: MD = −710.72, 95% CI −1217.05 to −204.38, P = 0.006; Type II: MD = −1126.26, 95% CI −1618.85 to −633.68, P = 0.0001; Figure S3D–S3F). Subgroup analysis that stratified studies based on the model of atrophy showed that bed rest, COPD, idiopathic scoliosis, and hip fracture induced a significant decrease in fibre CSA (Figure S3G–S3I).

**Myonuclear content following atrophy**
Analysis of 13 studies involving 260 participants found lower myonuclear content in response to skeletal muscle atrophy (mean: MD = −11, 95% CI −0.19 to −0.03, P = 0.005; Type I: MD = −0.09, 95% CI −0.17 to −0.00, P = 0.04; Type II: MD = −0.13, 95% CI −0.22 to −0.05, P = 0.003; Figure S3J–S3L). Interestingly, subgroup analysis showed myonuclear content in mixed, Type I, and Type II fibre only decreased in young adults and not in old adults who experienced atrophy (old adults: P = 0.61, P = 0.58, and P = 0.77, respectively). Subgroup analysis showed no difference between different period of interventions in mixed, Type I, and Type II fibres (P = 0.69, P = 0.81, and P = 0.64, respectively; Figure S3M–S3O). Stratifying studies based on the model of atrophy showed that bed rest, idiopathic scoliosis, and cerebral palsy induced a significant decrease in myonuclear content (Figure S3P–S3R).

**Myonuclear content in single muscle fibre following atrophy**
A single report with five astronauts assessed myonuclear content in single muscle fibre using 42–81 fibres from each biopsy sample before and after 11 days of space flight. This study reported no change in the myonuclear content of Type I fibres, whereas lower myonuclear content was found in Type II fibres.

**Myonuclear domain following atrophy**
Analysis of 10 studies involving 202 participants found a significant decrease in MND in response to skeletal muscle atrophy (mean: MD = −1.92, 95% CI −2.72 to −1.12, P = 0.00001; Type I: MD = −0.65, 95% CI −0.97 to −0.32, P = 0.0001; Type II: MD = −0.72, 95% CI −1.03 to −0.40, P = 0.0001; Figure S3S–S3W). The results from a single study with five astronauts showed lower MND in single muscle fibres after 11 days of space flight.

Subgroup analysis showed no difference between the reduction of MND in mixed, Type I, and Type II fibres in old and young adults and different periods of intervention (Figure S3X–S3Z). Stratifying studies based on the model of atrophy showed that leg immobilization, step reduction, cerebral palsy, and hip fracture induced a significant decrease in myonuclear content (Figure S3A–S3C).

**Satellite cell number following atrophy**
Analysis from 24 studies involving 611 participants found there was lower SC abundance in response to skeletal muscle atrophy (mean: SMD = −0.49, 95% CI −0.77 to −0.22, P = 0.0005; Type I: SMD = −0.20, 95% CI −0.59 to 0.20, P = 0.33; Type II: SMD = −0.37, 95% CI −0.71 to −0.02, P = 0.04; Figure S3U–S3V). In agreement with changes in myonuclear content, subgroup analysis showed SC content in mixed, Type I, and Type II fibre only decreased in young adults and not in old adults who experienced atrophy (old adults: P = 0.07, P = 0.76, and P = 0.35, respectively). Stratifying studies based on duration of the intervention period found that SC content was decreased after 60 days (mixed: MD = −0.85, 95% CI −1.61 to −0.09, P = 0.03; Type I: MD = −0.87, 95% CI −0.48 to −0.43, P = 0.01; Type II: MD = −1.02, 95% CI −1.61 to −0.44, P = 0.0006; Figure S3W–S3X). Stratifying studies based on the model of atrophy showed that bed rest, cerebral palsy, idiopathic scoliosis, and ACL injury induced a significant decrease in SC content (Figure S3A–S3C).

**Skeletal muscle responses in ageing compared with young adults**
Next, we assessed the impact of ageing on myonuclear content, MND, and SC abundance. Twenty-nine studies measured the aforementioned skeletal muscle characteristics in young and old adults. The details of the included studies are shown in Table 2.

**Myofibre size following ageing**
Analysis of 25 studies involving 724 participants found that except Type I fibres, CSA in mixed and Type II fibres decreased with ageing (mean: SMD = 0.91, 95% CI 0.25–1.56, P = 0.007; Type

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I: MD = −131.7, 95% CI −353.91 to 90.51, P = 0.25; Type II: MD = 1313.31, 95% CI 995.45–1631.16, P = 0.00001; Figure S4A–S4C).

**Myonuclear content following ageing** Analysis of 17 studies involving 494 participants found no change in myonuclear content of mixed and Type I fibres with ageing (MD = −0.03, 95% CI −0.24 to 0.19, P = 0.8; MD = −0.01, 95% CI −0.31 to 0.29, P = 0.95; respectively; Figure S4D and S4E). A pooled analysis from eight studies involving 274 participants found lower myonuclear content in Type II fibres with ageing (MD = 0.47, 95% CI 0.09–0.85, P = 0.02; Figure S4F).

**Myonuclear content in single muscle fibre following ageing** Two studies assessed muscle response to ageing at the single muscle fibre level. Cirstea et al. in a separate analysis of men and women reported a significant increase in myonuclear content of Type I fibres with no change in Type II fibres. In another study, Naro et al. reported no change in myonuclear content and MND of Type I and II fibres.

**Myonuclear domain following ageing** Analysis of 11 studies involving 346 participants found no change in MND of mixed and Type I fibres with ageing (MD = 236.01, 95% CI −11.78 to 483.79, P = 0.06; MD = −26.75, 95% CI −207.05 to 153.56, P = 0.77; respectively; Figure S4G and S4H). In contrast, there was lower MND in Type II fibres with ageing (MD = 296.19, 95% CI 109.08–483.29, P = 0.002; Figure S4I).

**Satellite cell number following ageing** Analysis of 25 studies involving 717 participants found lower SC abundance in mixed fibres with ageing (SMD = 0.78, 95% CI 0.37–1.19, P = 0.0002; Figure S4J). There was no change in SC content associated with Type I fibres, whereas SC content associated with Type II fibres was lower with ageing (SMD = 0.09, 95% CI −0.11 to 0.28, P = 0.38; SMD = 1.23, 95% CI 0.86–1.60, P = 0.00001; respectively; Figure S4K and S4L).

**Evidence from animal studies**

**Skeletal muscle responses to hypertrophy** Five studies assessed skeletal muscle growth in response to a hypertrophic stimulus induced by synergist ablation, weight loaded-ladder climbing, climbing, or weighted wheel running in extensor digitorum longus (EDL), flexor hallucis longus (FHL), plantaris, soleus, tibialis anterior (TA), and gastrocnemius muscles. Following exposure to an episode of overload-induced hypertrophy, skeletal muscle was subsequently exposed to disuse atrophy as a model of detraining or denervation. Given that young mice (<4 months old) have been shown to display a different response to overload-induced hypertrophy relative to mature mice (>4 months old), we performed a subgroup analysis to determine the effects of age on an episode of overload-induced hypertrophy followed by disuse atrophy on the aforementioned outcome variables. The details of the included studies are shown in Table 3.

**Myofibre size following training and detraining** Five studies assessed CSA response to increased activity. An episode of overload-induced hypertrophy significantly increased fibre CSA (SMD = 1.25, 95% CI 0.83–1.67, p = 0.00001; Figure S5A). Compared with control, there was no significant difference in fibre CSA after a detraining period (SMD = −0.60, 95% CI −1.71 to 0.51, P = 0.29), demonstrating that fibre CSA after a detraining period returns to baseline levels (Figure S5B). Subgroup analysis showed a significant difference between young and mature animals after training and detraining (P = 0.04 and P = 0.03, respectively), indicating that fibre CSA in young animals increases by a higher extent following training and decreases by a larger extent following detraining.

**Myonuclear content following training and detraining** Three studies assessed myonuclear content in muscle cross section. In response to a hypertrophic stimulus, there was a significant increase in myonuclear content (MD = 0.17, 95% CI 0.09–0.25, P = 0.0001; Figure S5C). Myonuclear content remained significantly elevated after a period of detraining compare with control animals (MD = 0.11, 95% CI 0.02–0.20, P = 0.01; Figure S5D). The number of studies was too small to permit subgroup analysis.

**Myonuclear content in single muscle fibre following training and detraining** Four studies assessed myonuclear content in single muscle fibre. An episode of overload-induced hypertrophy significantly increased myonuclear content (SMD = 2.26, 95% CI 1.28–3.23, P = 0.00001; Figure S5E). Myonuclear content following a period of detraining remained significantly elevated compared with control animals (SMD = 1.46, 95% CI 0.60–2.32, P = 0.0008; Figure S5F). Subgroup analysis of maturational age showed no statistically significant difference after overload-induced hypertrophy or detraining periods (P = 0.60 and P = 0.43, respectively).

**Skeletal muscle responses to atrophy** Eighty studies assessed skeletal muscle atrophy in response to different duration of denervation, hindlimb suspension, immobilization, space flight, tetrodotoxin blockade, and mechanical ventilation. We performed subgroup analyses to determine the potential impact that differences in the muscle under investigation, the duration of the intervention, and the model of atrophy used had on the atrophic response.

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Myofibre size following atrophy Analysis of 53 studies found lower CSA in response to skeletal muscle atrophy with a mean reduction of $\sim -36.9\%$ (SMD = $-1.96$, 95% CI $-2.21$ to $-1.71$, $P = 0.00001$; Figure S6A). Subgroup analysis for different muscles showed fibres CSA was significantly decreased in plantaris, soleus, gastrocnemius, pectoralis major, EDL, and TA (Figure S6B). Subgroup analysis that stratified studies based on duration of the intervention period ($\leq 5$ days, 7–14 days, 20–30 days, and $\geq 42$ days) found that myofibre CSA was decreased for all periods (Figure S6C). Subgroup analysis that stratified studies based on the model of atrophy showed that except for mechanical ventilation, all models induced a significant decrease in fibre CSA (Figure S6D). Subgroup analysis that stratified studies based on different methods of atrophy showed that myonuclear content was decreased in studies that performed hindlimb suspension, denervation, and immobilization (Figure S6E). Additionally, subgroup analysis based on %CSA reduction ($<20$, 20–29, 30–39, 40–49, and $>50$) showed that when %CSA reduction reach $\geq30$, myonuclear content decreased significantly (Figure S6F). A final subgroup analysis that stratified studies based on different methods of atrophy showed that SC content was decreased only in studies that performed hindlimb suspension ($\sim-24.4\%$) and immobilization ($\sim-30.1\%$). More interestingly, SC abundance was increased ($\sim+113.3\%$) in response to denervation (Figure S6G).

Myonuclear content following atrophy Analysis of 40 studies found lower myonuclear content in muscle cross section with a mean reduction of $\sim -20.6\%$ (SMD = $-1.03$, 95% CI $-1.30$ to $-0.76$, $P = 0.00001$; Figure S6H). Subgroup analysis that stratified studies based on different muscles showed that myonuclear content was decreased only in gastrocnemius, EDL, and soleus (Figure S6I). Subgroup analysis that stratified studies based on different intervention periods ($\leq 5$, 7–14, 20–30, and $\geq 42$ days) showed that myonuclear content was decreased in all periods (Figure S6J).

Myonuclear content in single muscle fibre following atrophy Analysis of 22 studies found lower myonuclear content in single muscle fibres with a mean reduction of approximately $-10.1\%$ (SMD = $-0.52$, 95% CI $-0.81$ to $-0.23$, $P = 0.0005$; Figure S6K). Subgroup analyses that stratified studies based on differences in muscle under investigation, duration of the intervention, and model of atrophy used found myonuclear content was only decreased in the soleus (Figure S6L). Subgroup analysis that stratified studies based on different intervention periods ($\leq 5$ days, 7–14 days, 20–30 days, and $\geq 42$ days) showed that myonuclear content was decreased in studies that lasted between 7–14 and more than 42 days (Figure S6M). Subgroup analysis that stratified studies based on different models of atrophy showed that myonuclear content was decreased in studies that performed hindlimb suspension and denervation (Figure S6N). Considering the different muscle type responses to atrophy, the discrepancy between the results for myonuclear content in whole muscle cross section and single muscle fibres may be due to the lower and selected fibre measurements in the studies that used single muscle fibre, as no more than 100 fibres were evaluated in any study.

Satellite cell number following atrophy Analysis of 41 studies found no change in SC content in cross-section (SMD = $-0.13$, 95% CI $-0.50$ to $-0.24$, $P = 0.48$) (Figure S6O). Subgroup analysis that stratified studies based on different muscles showed that SC content was decreased in soleus, whereas in TA it increased, and in EDL tend to increase (Figure S6P). Subgroup analysis that stratified studies based on different intervention periods ($\leq 5$, 7–14, 18–30, and $\geq 42$ days) showed a trend for lower SC content only in studies that lasted between 7 and 14 days (Figure S6Q).

Sensitivity analysis and publication bias

In regard to sensitivity analysis, the overall pooled estimates of the respective outcomes obtained in each analysis closely resembled the preliminary associations. Further, funnel plots were checked for the included studies in the meta-analysis, which suggested that in almost all analyses in human studies, there is no noticeable bias (Figure S7A–S7D). Additionally, Begg’s correlation rank and Egger’s regression did not show significant publication bias in almost all analyses in human studies (Table 4). In contrast, we found noticeable publication bias in most analyses of animal studies with significant Begg’s correlation rank and Egger’s regression results (Figure S7E and S7F; Table 4).

Discussion

The objective of the current systematic review and meta-analysis was to assess the myonuclear and SC content of either human or rodent skeletal muscle that had undergone hypertrophy, atrophy, or detraining. We found that both myonuclear and SC content in human skeletal muscle are lower with atrophy, ageing, and following a period of detraining; however, the change in myonuclear and SC content with detraining represents a return to pre-training levels. Subgroup analyses that stratified studies based on the age of the subjects showed that following detraining, Type I CSA in young adults decreases to a higher extent than in old adults. Additionally, following atrophy in human studies, we found that both myonuclear and SC content in mixed, Type I, and Type II fibres only decreased in young adults. In rodent studies, myonuclear content after an episode of overload-induced hypertrophy remains elevated during the subsequent detraining period. With atrophy in rodents,
Table 4 Meta-analysis of all studies

| Subgroup analysis                  | Classification | Heterogeneity | Model | Meta-analysis | P       | Beggs' P value | Eggers' P value |
|-----------------------------------|----------------|---------------|-------|---------------|---------|----------------|-----------------|
|                                   |                | P  | I² (%) |       | SMD (95% CI) |         |                |                |
| **Human studies: skeletal muscle responses to hypertrophy** |                |    |        |       |            |         |                |                |
| Outcome: CSA in whole cross section |                |    |        |       |            |         |                |                |
| Mixed fibre                       |                | 0.6 | 0%     | Fixed | 650.32 (355.30, 945.34) | 0.0001 | 1.0000         | 0.324           |
| After training                    |                |    |        |       |            |         |                |                |
| Type I fibres                     |                | 0.72 | 0%     | Fixed | 470.83 (168.29, 773.37) | 0.002  | 1.0000         | 0.618           |
| After detraining                  |                | 0.07 | 62%    | Random | 104.39 (604.46, 813.23) | 0.77   | 0.2963         | 0.309           |
| Type II fibres                    |                | 0.32 | 13%    | Fixed | 723.93 (358.02, 1089.84) | 0.0001 | 1.0000         | 0.363           |
| After detraining                  |                | 0.04 | 70%    | Random | 190.74 (882.92, 1264.40) | 0.73   | 0.2963         | 0.200           |
| **Outcome: Myonuclear content**   |                |    |        |       |            |         |                |                |
| Mixed fibres                      |                | 0.58 | 0%     | Fixed | 0.12 (0.00, 0.23) | 0.04   | 0.7341         | 0.491           |
| After training                    |                |    |        |       |            |         |                |                |
| Type I fibres                     |                | 0.70 | 0%     | Fixed | -0.14 (-0.26, -0.02) | 0.02   | 1.0000         | 0.993           |
| After detraining                  |                | 0.96 | 0%     | Fixed | -0.14 (-0.28, -0.00) | 0.05   | 1.0000         | -0.71           |
| Type II fibres                    |                | 0.4  | 0%     | Fixed | 0.23 (0.07, 0.40) | 0.006  | 1.0000         | 0.349           |
| After detraining                  |                | 0.61 | 0%     | Fixed | -0.23 (-0.37, -0.10) | 0.0009 | 1.0000         | 0.733           |
| **Outcome: Myonuclear domain**    |                |    |        |       |            |         |                |                |
| Mixed fibres                      |                | 0.55 | 0%     | Fixed | 43.16 (-42.14, 128.47) | 0.32   | 0.3082         | 0.349           |
| After training                    |                |    |        |       |            |         |                |                |
| Type I fibres                     |                | 0.34 | 0%     | Fixed | 5.67 (-133.51, 144.85) | 0.94   | IO             | IO              |
| After detraining                  |                | 0.42 | 0%     | Fixed | -9.26 (-166.29, 147.77) | 0.91   | IO             | IO              |
| Type II fibres                    |                | 0.8  | 0%     | Fixed | 73.87 (-52.35, 210.09) | 0.29   | IO             | IO              |
| After detraining                  |                | 0.48 | 0%     | Fixed | 55.98 (-138.18, 250.14) | 0.57   | IO             | IO              |
| **Outcome: Satellite cells**      |                |    |        |       |            |         |                |                |
| Mixed fibres                      |                | 0.52 | 0%     | Fixed | 0.75 (0.33, 1.18) | 0.0005 | 1.0000         | 0.814           |
| After training                    |                |    |        |       |            |         |                |                |
| Type I fibres                     |                | 0.84 | 0%     | Fixed | 0.16 (-0.32, 0.64) | 0.52   | 1.0000         | 0.808           |
| After detraining                  |                | 0.77 | 0%     | Fixed | 0.36 (-0.14, 0.85) | 0.16   | IO             | IO              |
| Type II fibres                    |                | 0.58 | 0%     | Fixed | -0.01 (-0.66, 0.65) | 0.99   | IO             | IO              |
| After detraining                  |                | 0.98 | 0%     | Fixed | 0.81 (0.30, 1.32) | 0.002  | IO             | IO              |
| **Human studies: skeletal muscle responses to atrophy** |                |    |        |       |            |         |                |                |
| Outcome: CSA                      |                |    |        |       |            |         |                |                |
| Mixed fibre                       |                | NA  | 61%    | Random | -497.24 (-734.13, -260.35) | 0.0001 | 0.0022         | 0.005           |
| Type I fibres                     |                | NA  | 62%    | Random | -735.16 (-1062.57, -407.75) | 0.0001 | 0.0369         | 0.089           |
| Type II fibres                    |                | NA  | 71%    | Random | -919.18 (-1292.14, -546.22) | 0.00001 | 0.0241         | 0.102           |
| **Outcome: Myonuclear content**   |                |    |        |       |            |         |                |                |
| Mixed fibre                       |                | NA  | 32%    | Fixed | -0.11 (-0.19, -0.03) | 0.005  | 0.0160         | 0.052           |
| Type I fibres                     |                | NA  | 0%     | Fixed | -0.09 (-0.17, -0.00) | 0.04   | 0.2129         | 0.141           |
| Type II fibres                    |                | NA  | 44%    | Fixed | -0.13 (-0.22, -0.05) | 0.003  | 0.1367         | 0.164           |
| **Outcome: Myonuclear domain**    |                |    |        |       |            |         |                |                |
| Mixed fibre                       |                | NA  | 72%    | Fixed | -1.92 (-2.72, -1.12) | 0.00001 | 0.7555         | 0.520           |
| Type I fibres                     |                | NA  | 0%     | Fixed | -0.65 (-0.97, -0.32) | 0.0001 | 0.5362         | 0.798           |
| Type II fibres                    |                | NA  | 0%     | Fixed | -0.72 (-1.03, -0.40) | 0.0001 | 0.3865         | 0.712           |
| **Human studies: skeletal muscle responses to atrophy** |                |    |        |       |            |         |                |                |
| Outcome: Satellite cells          |                |    |        |       |            |         |                |                |
| Mixed fibre                       |                | NA  | 61%    | Random | -0.49 (-0.77, -0.22) | 0.0005 | 0.0232         | 0.000           |
| Type I fibres                     |                | NA  | 71%    | Random | -0.20 (-0.59, 0.20) | 0.33   | 0.5289         | 0.081           |
| Type II fibres                    |                | NA  | 63%    | Random | -0.37 (-0.71, -0.02) | 0.04   | 0.4415         | 0.022           |

(Continues)
Table 4 (continued)

| Subgroup analysis | Classification                  | $I^2$ (%) | Model  | SMD (95% CI) | $P$   | Beggs’ $P$ value | Eggers’ $P$ value |
|-------------------|---------------------------------|----------|--------|--------------|-------|------------------|-------------------|
| **Human studies: Skeletal muscle responses in ageing compared with young adults** |                          |          |        |              |       |                  |                   |
| Outcome: CSA      | Mixed fibres                    | NA       | 63%    | Random       | 0.91  | 0.0135           | 0.107             |
|                   | Type I fibres                   | NA       | 42%    | Random       | −131.70 | 0.25             | 0.283             |
|                   | Type II fibres                  | NA       | 61%    | Random       | 1313.31 | 0.00001          | 0.189             |
| Outcome: Myonuclear domain | Mixed fibres | NA | 83% | Random | 236.01 | 0.06 | 0.8065 | 0.955 |
|                   | Type I fibres                   | NA       | 79%    | Random       | −26.75 | 0.77             | 0.646             |
|                   | Type II fibres                  | NA       | 75%    | Random       | 296.19 | 0.02  | 0.2655 | 0.502 |
| Outcome: Satellite cells | Mixed fibres | NA | 67% | Random | 0.78 | 0.0002 | 0.0179 | 0.006 |
|                   | Type I fibres                   | NA       | 79%    | Random       | 0.09  | 0.38             | 0.933             |
|                   | Type II fibres                  | NA       | 64%    | Random       | 1.23  | 0.00001          | 0.560             |
| **Animal studies: Skeletal muscle responses to hypertrophy** |                          |          |        |              |       |                  |                   |
| Outcome: CSA in whole cross-section | Mean CSA | Control vs training | 37% | Fixed | 1.25 | 0.00001 | 0.0163 | 0.091 |
|                   | Control vs detraining            | 85%      | Random | −0.60 (−1.71, 0.51) | 0.29 | 0.229 | 0.015 |
| Outcome: Myonuclear content in whole cross section | Mean CSA | Control vs training | 60% | Random | 0.17 | 0.0001 | 0.0894 | 0.149 |
|                   | Control vs detraining            | 59%      | Random | 0.11 (0.02, 0.20) | 0.01  | 0.0894 | 0.251 |
| Outcome: Myonuclear content in single muscle fibre | Mean CSA | Control vs training | 66% | Random | 2.26 | 0.00001 | 0.0085 | 0.062 |
|                   | Control vs detraining            | 68%      | Random | 1.46 (0.60, 2.32) | 0.0008 | 0.0085 | 0.033 |
| **Animal studies: Skeletal muscle responses to atrophy** |                          |          |        |              |       |                  |                   |
| Outcome: CSA in whole cross-section | Mean CSA | NA | 63% | Random | −1.96 (−2.21, −1.71) | 0.00001 | 0.0000 | 0.000 |
| Outcome: Myonuclear content in whole cross section | Mean CSA | NA | 65% | Random | −1.03 (−1.30, −0.76) | 0.00001 | 0.0000 | 0.000 |
| Outcome: Satellite cells in whole cross section | Satellite cells | NA | 81% | Random | −0.13 (−0.50, 0.24) | 0.48 | 0.5724 | 0.266 |
| Outcome: Myonuclear content in single muscle fibre | Mean CSA | NA | 62% | Random | −0.52 (−0.81, −0.23) | 0.0005 | 0.0000 | 0.000 |

CSA, cross-sectional area; IO, insufficient observation; NA, not applicable; SMD, standard mean difference.
myonuclear content is sensitive to the muscle type and the model of atrophy. More interestingly, we found that in animals, an atrophy of myofibre CSA of ≥30% was associated with a significant decrease in myonuclei. Skeletal muscle fibres have a memory of prior chronic contractile activity, termed ‘muscle memory’. Evidence suggests myonuclei acquired during an initial period of hypertrophy are associated with enhanced muscle growth upon resumption of training following a period of detraining.16,17 An obvious, but debated, critical aspect of this proposed mechanism of atrophy in humans is the ‘new’ myonuclei must be retained throughout the period of detraining.16,17 The present meta-analysis found that exercise-induced myonuclei were not retained during detraining in humans but were in rodents. The rodent finding should be viewed with some caution as only five studies were included in the analysis with one study using denervation as a model of detraining following synergist ablation-induced hypertrophy.16 The concern with denervation as a model of detraining stems from our meta-analysis showing that denervation in rodent skeletal muscle causes a significant increase in SC content. Thus, it is not clear if the elevated myonuclear content reported by Bruusgaard et al.16 after denervation-induced atrophy was driven by enhanced SC fusion, which would mask any loss of myonuclei. Other concerns that need to be taken into consideration are the magnitude of the hypertrophic response and the age of animals. The 25–60% increase in skeletal muscle CSA in response to synergist ablation571–575 is much higher than 6–10% increase in quadriceps CSA in response to resistance training in humans.575–579 Furthermore, three of the five rodent studies used animals under 4 months old.16,18,92 Considering the different SC requirements for hypertrophic growth in fully mature mice compared with juvenile mice,93 the elevated myonuclear content during detraining might reflect a low level of SC fusion known to occur in juvenile mice.580 Additional animal studies are needed to more definitively answer the question of whether or not myonuclei acquired during hypertrophy are permanent during periods of detraining. Moreover, evaluating the same muscle in human studies directly assessed muscle memory showed no change in myonuclear number with atrophy of 10%; however, when atrophy was ≥30% in rodents, myonuclear content was lower. These findings reveal that, in rodents, myonuclear content is stable, except under the most extreme atrophic conditions.22 Needing more evidence in both humans and rodents, we decided to assess myonuclear content and SC numbers after exposure to atrophy. The results of our meta-analysis showed that myonuclear content and SCs of atrophied human Type II fibres decrease following atrophy. This analysis also found that myonuclear content in rodents decreases in response to hindlimb suspension, denervation, and immobilization with SC content lower in response to hindlimb suspension and immobilization. These findings implicate that myonuclear and SC content in both humans and rodents are not maintained indefinitely and may be reduced with skeletal muscle atrophy. Interestingly, we found lower myonuclear content was associated with higher SC content in response to denervation. These results can be explained by a higher rate of atrophy and a lower rate of myonuclear reduction in response to denervation (44 vs. 16%, respectively) compared with hindlimb suspension (35 vs. 25%, respectively), and immobilization (28 vs. 19%, respectively). Finally, needing more evidence regarding the possibility of long-term myonuclear permanence in humans, we assessed...
myonuclear content, MND, and SC numbers in studies that compared young and elderly adults. Interestingly, we found that human ageing is accompanied by reduction in myonuclear content, MND, and SC abundance in atrophied Type II myofibres. These results clearly demonstrate that myonuclei are not retained indefinitely throughout the human lifespan.

To better understand how skeletal muscle possesses a memory of prior chronic contractile activity, recent studies have focused on the potential role of epigenetics. Skeletal muscle may possess a long-term DNA hypomethylation ‘memory’ of prior exercise training that could have consequences for future myofibres adaptability during retraining.94–96 Future studies should evaluate the role of epigenetic ‘memory’ association with a first training period to extend our understanding of the molecular bases of ‘muscle memory’.

**Limitations**

There are several limitations of the systematic review and meta-analysis. First, despite the intense interest in the concept of ‘muscle memory’, the evidence to support the concept remains anecdotal as illustrated by the paucity of human and animal studies (i.e. only five studies in animals and four studies in humans). Second, different muscles were analysed across the animal studies, which confounded the results. Third, the different rates of muscle hypertrophy and myonuclear accretion between humans and animals make it quite challenging to translate animal results to in vivo human setting. Fourth, the small number of human studies made it challenging to determine the relationship between myonuclear content and the degree of atrophy as observed in rodents. Fifth, the analysis of SC content during atrophy in human studies associated with different diseases or models of atrophy was unable to identify a loss of SC content is related to a particular disease state or model of atrophy. Sixth, the current meta-analysis is based on the assumption that all studies accurately measured myonuclear content. To accurately quantify myonuclear abundance by muscle cross section (which represents the vast majority of the studies analysed), it is critical to clearly identify the myofibre cell border; yet this approach can be hampered by the fact that a three-dimensional structure, that is, the myofibre is being assessed in two dimensions.

This can lead to the mis-identification of a satellite cell nucleus being inside the myofibre or, alternatively, a bona fide myonucleus not being counted as it appears outside the dystrophin border. While this scenario is possible, it is assumed to have a minor impact, if at all, on the quantification of myonuclear content. We generated a new transgenic mouse model that allows for the definitive identification of myonuclei via nuclear GFP-labelling, which should help to further minimize this inherent limitation of quantifying myonuclear content by muscle cross section.97 Finally, the meta-analysis of animal studies should be interpreted with caution as publication bias may be present.

**Conclusion**

The findings of this study extend and add new information to the field’s knowledge regarding the concept of ‘muscle memory’ based on the idea that, once myonuclei are acquired, they are permanent. In humans, myonuclear content is not stable as it was found to change in response to a bout of detraining or atrophy. This finding suggests that other mechanisms are operative in mediating muscle memory. In rodents, the stability of myonuclei is less clear because of the limited number of studies and differences in experimental design across studies.

**Conflict of interest**

The authors declare that they have no conflicts of interest relevant to the content of this review.

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**Online supplementary material**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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