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OPINION

Muscle memory theory: A critical evaluation

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Introduction

From a cellular perspective in the context of muscle growth, 'muscle memory' can be described as elevated responsiveness to a hypertrophic stimulus as a consequence of having experienced such a stimulus previously. 'Muscle memory theory' would thus imply that having previously been exposed to a pharmacological growth stimulus (e.g. anabolic steroids) and/or loading (e.g. resistance training) would predispose the muscle to grow more rapidly and/or in greater magnitude to a second growth-promoting exposure after a washout period (e.g. cessation of steroid usage and/or training). In our opinion, the question is not whether muscle memory exists as described – there are numerous examples for it in the literature (Seaborne et al., 2018; Sharples & Turner, 2023; Wen et al., 2021) – but, instead, what is the mechanism controlling it? The most publicised mechanistic explanation for how skeletal muscle stores memory and exploits it upon re-exposure to a stimulus is 'nuclear permanence' (Sitko et al., 2025). Briefly, as a skeletal muscle grows, muscle fibre nuclei (myonuclei) are added to multinuclear muscle fibres via tissue resident muscle stem cells (called satellite cells). This myonuclear addition theoretically supports muscle fibre synthetic capacity for hypertrophy as each myonucleus is thought to govern a finite amount of cytoplasmic volume, called the 'myonuclear domain' (Bagley et al., 2023).

The muscle memory theory emerged from the contention that myonuclei, once added to a growing muscle fibre, are never subsequently removed, even after a fibre returns to its original size (Gundersen, 2016). Anabolic steroids in isolation but particularly in combination with resistance-type training can drive excessive muscle growth and commensurate myonuclear addition to adult muscle fibres. Because of the high level of myonuclear accretion from training and anabolic steroid usage, there is speculation as to whether previously hypertrophied muscle fibres with abnormally high myonuclear density (i.e. hypernucleated) could confer long-term adaptive advantages (Sitko et al., 2025). The most obvious implication of hypernucleated muscle fibres is that a former steroid user could be banned from athletic competition because of muscle memory conferred by myonuclear permanence. With the severity of such a punishment, it is imperative to have a firm but also nuanced understanding of the evidence for myonuclear permanence as a mechanism for muscle memory. There are also clinical implications for understanding muscle memory mechanisms as it may help inform the timing of rehabilitation interventions. This article is a response to a recent Opinion piece in *The Journal of Physiology* by Sitko et al. (2025). We seek to provide perspectives and alternative hypotheses on muscle memory theory and its possible implications, with a focus on experimental considerations and contemporary understanding of myonuclear biology.

Muscle memory theory: more than meets the eye. The genesis of muscle memory theory can be traced to an influential article by the Gundersen laboratory published in 2013 (Egner et al., 2013). Egner et al. (2013) sought to determine the lasting effects of anabolic steroid use on myonuclear permanence and muscle memory, building on previous work indicating that myonuclei acquired during hypertrophy are not lost during denervation-induced atrophy in rats in the short-term (i.e. weeks) (Bruusgaard et al., 2010). In brief, female mice of undisclosed age were treated with testosterone to increase muscle mass and myonuclear number. A 3-month cessation from testosterone caused muscle fibres to return to their initial pre-steroid exposure size, yet myonuclear number (and therefore density) was reportedly elevated. Then, muscle fibres after steroid cessation were mechanically overloaded using a surgical technique. Hypernucleated muscle fibres in the steroid-treated muscles had a greater rate and magnitude of muscle fibre hypertrophy after 2 weeks of overload relative to non-steroid controls. The exaggerated hypertrophic response in the steroid group was attributed to beginning the overload period with more myonuclei.

Data in Egner et al. are provided from two independent methods showing that myonuclei were not lost after cessation from exogenous supraphysiological anabolic steroids: *in vivo* imaging, which is innovative but limited to analysing the myonuclei of a few fibres on the surface of a muscle; and Hoechst/dystrophin histology – a widely used and generally validated approach (Egner et al., 2013). However, a closer inspection of the histological data raises questions as to the veracity of the conclusion that myonuclei are permanent after steroid exposure. Myonuclear number in the extensor digitorum longus (EDL) muscle is ∼0.7 myonuclei per fibre cross-section in controls (no steroid) and ∼1.0 myonuclei per fibre cross-section immediately following testosterone treatment. Following a 3 month cessation from testosterone (∼12% of murine lifespan), myonuclei per fibre of the EDL is ∼0.8. This is an ∼20% lower value, which implies a non-trivial loss of myonuclei in this muscle. Such an observation leads to several important questions germane to the current discussion: (1) how long are acquired myonuclei retained in adult skeletal muscle fibres? (2) What methodological considerations exist when measuring myonuclear number? (3) Are there other sources of muscle memory unrelated to elevated myonuclear density?

Myonuclear permanence: of mice and men. Brief unloading periods characterized by muscle fibre atrophy in rodents (days to weeks) and humans (weeks to a few months) is typically not associated with a lower number of myonuclei (Jackson et al., 2012; Schwartz & Gundersen, 2022a; Schwartz & Gundersen, 2022b; Sitko et al., 2025); however, there are some exceptions (Allen,

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Linderman, Roy, Grindeland et al., 1997; Gallegly et al., 2004; Hikida et al., 1997), which we have discussed at length previously (Murach et al., 2018). Over longer periods, there is mounting evidence that myonuclei are lost during muscle atrophy from unloading, inactivity and/or ageing across species (Bagley et al., 2023; Murach et al., 2018; Rahmati et al., 2022). There is also strong evidence to suggest that severe atrophy induced by spinal cord injury or paralysis causes myonuclear loss (Dupont-Versteegden et al., 1999; Viggars, Wen et al., 2022). Evidence for myonuclear loss across conditions has been discussed in detail recently (Bagley et al., 2023; Kirby & Dupont-Versteegden, 2022; Kirby & Dupont-Versteegden, 2022).

The mechanism(s) of myonuclear removal are hotly debated but not well-understood (Kirby & Dupont-Versteegden, 2022; Kirby & Dupont-Versteegden, 2022; Schwartz & Gundersen, 2022a; Schwartz & Gundersen, 2022b). Myonuclear apoptosis or autophagy are possible explanations. Substantiation for these mechanisms is generally limited, but signs of myonuclear apoptosis have been reported in skeletal muscles after immobilization (Smith et al., 2000; Tanaka et al., 2022), unloading (Allen, Linderman, Roy, Bigbee et al., 1997; Dupont-Versteegden et al., 2006), mechanical ventilation-induced atrophy (McClung et al., 2007), cancer cachexia (Baltgalvis et al., 2010) and ageing (Siu et al., 2005), as well as in extraocular muscles that may experience high myonuclear turnover (McLoon et al., 2004). Classic work from Bruce Carlson's laboratory involved detailed and meticulous analysis of denervated muscle fibres using high-resolution microscopy (Dedkov et al., 2001; Lu et al., 1997; Viguie et al., 1997). This work showed progressive lowering of myonuclear number with denervation over time, myonuclei featuring obvious signs of degeneration, and muscle fibres with large regions devoid of myonuclei (Borisov & Carlson, 2000; Dedkov et al., 2001; Lu et al., 1997; Viguie et al., 1997). Myonucleus-free regions of muscle fibres were attributed to myonuclear 'death' and/or myonuclear translocation (Viguie et al., 1997). Signs of myonuclear degeneration during denervation has been observed using contemporary techniques as well (Adhihetty et al., 2007). Apoptotic signalling is a rapid process and can be difficult to capture using 'snapshot' measures; however, absence of evidence is not evidence of absence that a potent and conserved myonuclear removal mechanisms exists across conditions. Mitochondria, which have their own genome, are routinely removed in health and disease from muscle fibres via specialized mechanisms such as mitophagy (Leduc-Gaudet et al., 2021), and the same could be true for myonuclei (Luo et al., 2016).

Regarding myonuclear loss, a similar trend to unloading and inactivity emerges with respect to detraining from hypertrophic exercise training. After 3 months (∼12% of murine lifespan) or more of detraining following 8 weeks of hypertrophic progressive weighted wheel running (PoWeR) in adult female mice (training starting at >4-month-old), myonuclear numbers in the plantaris and gastrocnemius hindlimb muscles were significantly lower (∼20%) concomitant with a return to pre-training muscle fibre size (Dungan et al., 2019; Murach et al., 2020). This finding was confirmed using two independent analyses: (1) DAPI/dystrophin histology of entire muscle cross-sections using unbiased semi-automated imaging analysis and (2) myonuclear counts on isolated single muscle fibres fixed *in situ* at resting length and normalized to fibre segment length. Using these two techniques, myonuclei of the soleus muscle were not lower after 6 months of detraining (∼1/4 of mouse lifespan) following PoWeR in mice, but the soleus muscle also did not detrain according to fibre size or type distribution (Murach et al., 2020). Oxidative type 2 fibres of the gastrocnemius muscle did detrain and had lower myonuclear counts after detraining. Fibre type- and muscle type-specific considerations for myonuclear permanence is under-studied and deserve further consideration. Longitudinal studies of similar duration relative to lifespan as in animal studies are difficult if not impossible to execute in humans. Findings of shorter duration are therefore conducted, and the conclusions are then extrapolated.

One recent study aimed to test muscle memory after 16 weeks of detraining following resistance training in humans (Psilander et al., 2019). Despite muscle fibre hypertrophy, vastus lateralis muscles of humans did not experience myonuclear accretion during the initial training measured by two independent techniques: histology and isolated single fibre myonuclear counts from muscle biopsy samples (Psilander et al., 2019). This study therefore did not address the permanency of myonuclei after detraining (Psilander et al., 2019). With the relatively large sample size in this study, however, a re-analysis showed that the subset of subjects who responded as expected, with myonuclear accretion during the initial training, lost myonuclei upon detraining (Murach et al., 2019). The myonuclear counts per fibre of the 'responders' was within normal ranges in reference to the broader literature (Karlsen et al., 2015) and not considered extreme, meaning that 'regression to the mean' may not explain myonuclear loss with detraining (Eftestøl et al., 2020; Snijders et al., 2020). Furthermore, careful muscle histology and single fibre analysis for myonuclear density across time points should not yield results that are so unreliable that regression to the mean is an explanation for the findings. This re-analysis also reinforced the possible degree of agreement between histological and isolated single muscle fibre myonuclear counts. The patterns for these two measures mirrored each other across individuals and time points almost perfectly (Murach et al., 2019). Another recent longitudinal study using muscle histology in humans suggested that myonuclei are lost after an extended period of detraining (Snijders et al., 2019). In their study, Snijders et al. (2019) resistance trained older adults for 6 months, then evaluated changes in vastus lateralis muscle fibre size and myonuclear density following a year of training cessation. Hypertrophy of type 2 muscle fibres ensued with training concomitant with increased myonuclear density, both of which returned to baseline levels after a year of detraining (Snijders et al., 2019). It is worth notingthat Snijders et al. (2019) used laminin to identify myonuclei, and it is not clear whether satellite cells were accounted for in the myonuclear counts. Blocquiaux et al. (2020) reported similar trends to Snijders et al. (2019) with 12 weeks of detraining in older individuals after resistance training then detraining using histology. Collectively, if myonuclei are quantified after a longer period of detraining in rodents and humans they appear to be lost, in agreement with the broader literature after prolonged atrophy in other contexts (Rahmati et al., 2022). A summary of select studies reporting myonuclear loss across species and conditions is shown in Table 1. The time course of myonuclear loss – specifically in the context of doping offenses – could have implications for suspension duration if elevated myonuclear number is a source of muscle memory.

Table 1. Examples of studies reporting myonuclear loss after a period of detraining/unloading using dystrophin histology and/or single muscle fibre myonuclear counts

Reference	Species	Age	Sex	Myonuclear detection method(s)	Method details	Muscle(s) analyzed	Intervention and duration	Myonuclear counts per fibre
Studies reporting myonuclear loss with training/detraining or atrophy								
Dupont-Versteegden, EE et al. (1999)	Rat	'Adult'	Female	Dystrophin and Hoechst	Cross-sections (ROI, 70–100 fibres)	Soleus	10 days spinal cord transection	↓ 25%
Dupont-Versteegden, EE et al. (2000)	Rat	'Adult'	Female	Dystrophin and Hoechst	Cross-sections (ROI, 70–100 fibres)	Soleus and Plantaris	8 weeks spinal cord transection	↓ 40%
Gallegly, JC et al. (2004)	Rat	6, 20, 32 months	Male	Dystrophin and Hoechst	Cross-section (ROI, 250–300 fibres)	Soleus	1- and 2-weeks hind limb suspension	↓ ∼30%
Leeuwenburgh, C et al. (2005)	Rat	6 months and 32 months	Male	Dystrophin and Hoechst	Cross-section (ROI, 120–150 fibres)	Soleus	2 weeks hind limb suspension	↓ ∼30%
Dungan, CM et al. (2019)	Mouse	≥4 months	Female	Dystrophin and DAPI Single fibre myonuclear counts	Cross-sections (entire muscle) and single fibres: 30 fibres per muscle per mouse	Plantaris	12 weeks detraining from hypertrophic PoWeR training	↓ 18%
Murach, KA et al. (2020)	Mouse	≥4 months	Female	Dystrophin and DAPI Single fibre myonuclear counts	Cross-sections (entire muscle) and single fibres: five from soleus and 10 from gastroc per muscle per mouse	Soleus Gastroc	24 weeks detraining from hypertrophic PoWeR training	Gastroc = ↓ ∼15% Soleus = No loss, but no signs of atrophy

Abbreviations: Gastroc, gastrocnemius; ROI, region of interest

The devil is in the (methodological) details. Understanding whether myonuclei are permanent after being added to a muscle fibre relies on accurate quantification of myonuclei. Measuring myonuclei in skeletal muscle is challenging since interstitial nuclei – outside of the muscle fibre but in close anatomical relationship – must be excluded. Typically, DAPI or Hoescht (DNA/nuclei) and dystrophin (muscle fibre membrane) via histology are used. This analysis can be subjective because data from DNA stains and dystrophin immunodetection on muscle cross-sections are contingent on the quality of muscle sample processing, subsequent immunodetection/staining, skill with imaging, manner of analysis (e.g. manual counting or unbiased software) and number of fibres analysed. These factors can vary widely between laboratories. Isolated single muscle fibres that are stripped of adherent interstitial cells are a gold-standard measure for myonuclear counts, but this method is tissue-intensive, requiring specialized microscopy techniques and consideration for sarcomere spacing. Nevertheless, as we mention above, DNA staining with dystrophin and myonuclear counts on isolated muscle fibres can reflect one another closely when performed carefully (Dungan et al., 2019; Murach et al., 2019; Psilander et al., 2019). It is worth emphasizing that to detect a relatively modest 5–15% difference in myonuclear density across time points or conditions, an appropriately powered sample size should be used.

An important but often overlooked component of analysing myonuclear density via histology is that myonuclear length can become appreciably longer or shorter with exercise training (Battey et al., 2023; Murach et al., 2019) and ageing (Cristea et al., 2010) in rodents and humans. As such, histological measures of myonuclear counts can be misleading depending on training status and/or age, which should be accounted for. An antibody (anti-PCM1) that can be specific to myonuclei under resting/non-stressed conditions may help with the accurate quantification of myonuclei (Winje et al., 2018), but PCM1 detection becomes non-specific in skeletal muscle under stress (Viggars, Owens et al., 2022). Therefore, any study evaluating myonuclear gain or loss must be evaluated through the lens of these method-related considerations and caveats. Multiple confirmatory methods are preferred. In rodent studies, the age of the animals may be an important consideration regarding myonuclear addition and removal. Myonuclear addition to support hypertrophy could be more consequential in young growing mice as opposed to mature/adult mice (McCarthy et al., 2017; Murach et al., 2017). The permanency of myonuclei added during hypertrophy could depend on this initial age-dependent requirement of myonuclear addition for adaptation. Several muscle memory studies have been carried out in young growing/juvenile rodents or the age of the animals was not disclosed (Bruusgaard et al., 2010; Eftestøl et al., 2022; Egner et al., 2013; Lee et al., 2018); these study design considerations could complicate interpretation of results if developmental age influences myonuclear permanence. Overall, decisions on whether an athlete should be expelled from sport based on the potential ergogenic effects of enhanced myonuclear number need to be approached with caution given the current state of the literature, the nuanced nature of myonuclear counting in skeletal muscle samples and details of study designs.

Cellular muscle memory: more than a numbers game? It is conceivable that hypernucleated muscle fibres confer a short-term advantage for more rapid and/or amplified muscle hypertrophy upon retraining. However, if myonuclei are not permanent – regardless of whether they are acquired by anabolic steroids and/or resistance training – what else could explain long-term 'muscle memory'? Studies in mice and humans suggest that exaggerated hypertrophy upon retraining is explained by persistent adaptations to the skeletal muscle methylome, transcriptome and proteome (Figure 1) (Hulmi et al., 2025; Seaborne et al., 2018; Wen et al., 2021). Seaborne et al. (2018) reported an epigenetic memory after resistance training in human muscle tissue that may have facilitated retraining adaptations. Specifically, there was a persistent change to UBR5 promoter region hypomethylation following detraining from resistance training that related to exaggerated UBR5 gene expression upon retraining. UBR5 was later shown mechanistically to control muscle mass (Hughes et al., 2021; Seaborne et al., 2019). Our laboratory reported that changes to DNA methylation of growth-related genes (e.g. *Pkd2l1*, *Ski* and *Eif1a*) specifically in resident myonuclei after hypertrophic exercise training remain after detraining and could contribute to more rapid regrowth upon retraining (Wen et al., 2021). New and emerging technologies such as single nucleus RNA-sequencing and spatial RNA-sequencing in muscle could provide deeper insights into the molecular features of unique resident myonuclei that may help drive re-growth after detraining. Using proteomics, a recent investigation in human skeletal muscle showed that carbonyl reductase 1 (CBR1) and proteins involved in muscle contraction, the cytoskeleton, and calcium binding (e.g. CAPN2) were persistently elevated after 10 weeks of detraining (Hulmi et al., 2025). Other evidence in rodents suggests that long-term changes to miRNAs – specifically the highly-abundant muscle fibre-enriched miR-1 – could contribute to muscle memory (Murach et al., 2020). The myomiR mir-1 has been implicated in regulating hypertrophic processes via the induction of 'Warburg' or 'aerobic glycolysis' metabolism to support growth (Fei et al., 2025; Ismaeel et al., 2025; Valentino et al., 2021). Most alternative mechanisms of 'muscle memory' have not been tested in the context of anabolic steroid usage, and so further investigation is required. Finally, it is possible that other cellular and morphological features of skeletal muscle apart from myonuclear number, such as an abnormal proportion of centralized/misplaced myonuclei and/or muscle fibres with the appearance of 'splitting' (probably *de novo* adult myogenesis) could serve as a biomarker for prior anabolic steroid usage (Elgendy et al., 2018). Collectively, these findings suggest that muscle memory could be about more than a myonuclear 'numbers game'. Understanding the basic mechanisms of muscle memory will provide new directions on the biology of doping detection, as well as help inform countermeasures against age-related muscle atrophy and loss of plasticity to exercise.

Conclusions

Muscle memory has broad implications for muscle biology, as well as usefulness in determining fair play in sport, and also has implications for muscle plasticity across the lifespan. Because of the important consequences of muscle memory, it requires a deep understanding of myonuclear biology and measurement approaches.

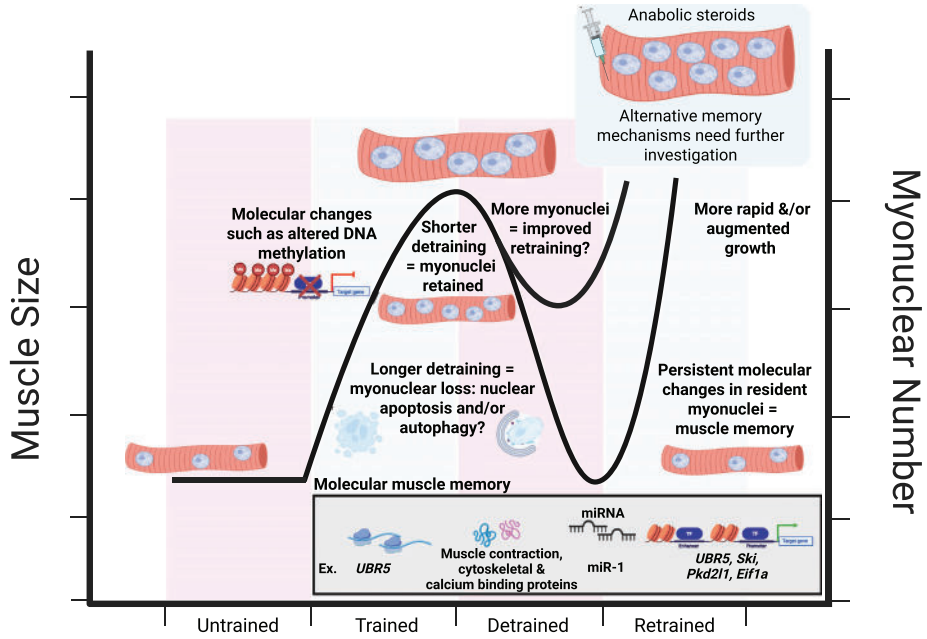

Figure 1. Muscle memory theory
Summary of 'muscle memory theory' in the context of myonuclear addition and removal after training and detraining, with consideration of possible alternative mechanisms

Careful inspection of published work suggests that myonuclear density is diminished following unloading or a period of detraining. Myonuclear permanence therefore may fail to uphold the theory of muscle memory. Instead, we point to the potential for lasting adaptations in the skeletal muscle methylome, proteome, and transcriptome that may be related or unrelated to myonuclear number. Future research should seek to address methodological concerns by incorporating multiple confirmatory methods in determining myonuclear density, as well as expanding the search for muscle memory mediators beyond myonuclear density.

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Additional information

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No competing interests declared.

Author contributions

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Supporting information

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