Received: 30 September 2019 | Revised: 5 March 2020 | Accepted: 10 March 2020

DOI: 10.1111/apha.13465

REVIEW ARTICLE

ACTA PHYSIOLOGICA

The concept of skeletal muscle memory: Evidence from animal and human studies

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Abstract

Within the current paradigm of the myonuclear domain theory, it is postulated that a linear relationship exists between muscle fibre size and myonuclear content. The myonuclear domain is kept (relatively) constant by adding additional nuclei (supplied by muscle satellite cells) during muscle fibre hypertrophy and nuclear loss (by apoptosis) during muscle fibre atrophy. However, data from recent animal studies suggest that myonuclei that are added to support muscle fibre hypertrophy are not lost within various muscle atrophy models. Such myonuclear permanence has been suggested to constitute a mechanism allowing the muscle fibre to (re)grow more efficiently during retraining, a phenomenon referred to as "muscle memory." The concept of "muscle memory by myonuclear permanence" has mainly been based on data attained from rodent experimental models. Whether the postulated mechanism also holds true in humans remains largely ambiguous. Nevertheless, there are several studies in humans that provide evidence to potentially support or contradict (parts of) the muscle memory hypothesis. The goal of the present review was to discuss the evidence for the existence of "muscle memory" in both animal *and* human models of muscle fibre hypertrophy as well as atrophy. Furthermore, to provide additional insight in the potential presence of muscle memory by myonuclear permanence in humans, we present new data on previously performed exercise training studies. Finally, suggestions for future research are provided to establish whether muscle memory really exists in humans.

KEYWORDS

muscle adaptation, muscle memory, myonuclear domain size, myonuclei, satellite cell

1 | INTRODUCTION

Skeletal muscle fibres are large multinucleated cells, with every muscle fibre containing hundreds to thousands of nuclei. For example, a single human biceps muscle fibre of 10 cm in length contains about 3000 nuclei.[1] Whereas in most cells the nucleus occupies the centre of the cell body, within muscle fibres the nuclei are positioned peripherally adjacent to the plasma membrane. Only under certain conditions like development, repair/regeneration, or specific pathologies,

nuclei can be found migrating to the centre of a muscle fibre. Myonuclei are post-mitotic, and have a flattened and elongated shape with the long-axis typically running parallel to the longitudinal axis of the muscle fibre. Nuclei are not randomly distributed within the muscle fibre. In fact, nuclei appear to repel each other during positioning resulting in an evenly distributed configuration within the muscle fibre.[2] As early as the 19th century,[3] it was hypothesized that every nucleus holds jurisdiction over a certain volume of the muscle fibre cytoplasm, initially referred to as the "karyoplasmatic" ratio. More recently, the concept of a muscle fibre being divided into evenly distributed compartments, each under the control of a single myonucleus, has been referred to as the "DNA unit" [4] or "myonuclear domain".[5] According to the myonuclear domain theory, every nucleus has a limited transcriptional capacity, only synthesizing proteins for use in the immediate vicinity surrounding the nucleus.[5,6]

Based on the concept of the myonuclear domain theory, an approximate linear relationship must exist between total myonuclear number and muscle fibre size and/or volume. Although the myonuclear domain theory was quickly adopted by many investigators, it continues to be intensely debated within the field.[7-14] For instance, based on the paradigm, the myonuclear domain should be kept (relatively) constant by adding additional nuclei (supplied by muscle satellite cells) during muscle fibre hypertrophy and by nuclear loss (through apoptosis) during muscle fibre atrophy.[14] However, more recent animal studies demonstrated that myonuclei do not appear to be lost with various muscle atrophy models.[7] Furthermore, it has been hypothesized that myonuclei that are added to support muscle fibre hypertrophy are not lost during detraining.[15,16] Such a "myonuclear permanence" could constitute a mechanism allowing the muscle fibre to grow more efficiently during retraining, as the myonuclear number would remain in an elevated or "trained" state. This potential phenomenon is referred to as "muscle memory".[8] The proposed existence of a "muscle memory" to hypertrophic stimuli may hold a number of consequences across athletic and clinical settings. For instance, anabolic steroids are used across a variety of elite sporting competitions to induce muscle growth and enhance recovery. In this setting, the ability to permanently add nuclei to muscle fibres may create an unfair competitive advantage to previously caught and suspended doping offenders when returning back to competition. However, muscle memory may also promote quicker muscle (re)growth in older adults who participated in resistance-type exercise training earlier in life, potentially providing a clinical benefit when aiming to combat age-related muscle loss. Importantly though, the "muscle memory" hypothesis has mainly been based on data attained from experimental rodent models. As clear differences in muscle architecture and metabolism exist between species,[17,18] translating these results to humans is challenging. Thus far, any evidence from human studies to either support or refute the muscle memory hypothesis has largely been ignored. The goal of the present review was to showcase any evidence for the presence or absence of the proposed "muscle memory" in both animal and human models of muscle fibre atrophy and hypertrophy. To provide additional insight on the potential presence of muscle memory by myonuclear permanence in vivo in humans, we have re-analysed some of our previously performed exercise training studies. Finally, we provide suggestions for future research to establish whether muscle memory exists in humans.

2 | CURRENT EVIDENCE FROM ANIMAL STUDIES

According to the original hypothesis of a constant myonuclear domain, myonuclei are added during muscle fibre growth and lost during muscle atrophy.[14] In contrast, the muscle memory theory postulates that myonuclei are never lost from the skeletal muscle fibre, resulting in a reduction in myonuclear domain size during muscle fibre atrophy. A large number of animal studies, however, have reported a reduction in myonuclear number in atrophy-inducing models such as denervation,[19-22] spinal cord transection,[23-25] mechanical unloading[26-30] and spaceflight[31-33] (see Table 1). Importantly though, many of these studies have used muscle cross-sections to count myonuclei, and the determination of myonuclear content and apoptosis within muscle cryosections is not without uncertainty.[7,15,34] Using conventional histology, in which nuclei are labelled (eg DAPI or Hoechst dye) for light or fluorescent microscopy on muscle cross sections, there may be difficulties to distinguish true myonuclei from other nuclei, in particular muscle satellite cells. Until recently,[35] no antibody was available that specifically stains for myonuclei. Therefore, a staining approach in which a nuclear stain is combined with specific antibodies that delineates the muscle cell border would be an absolute requirement to allow clear distinction of nuclei located in or outside the muscle fibre. Staining against laminin or dystrophin protein is currently most frequently used to visualize muscle fibre cell borders. Some have suggested that staining of dystrophin may be more accurate in the assessment of myonuclear content than laminin,[36,37] however, this remains to be more firmly established in both rodent as well as human skeletal muscle cross-sections. Besides delineation of the muscle fibre border, it is critical to exclude muscle satellite cells from the myonuclear count as these cells are also located inside (between sarcolemma and basal lamina) the muscle fibre. Although muscle satellite cells account for only 2%-4% of total myonuclei within muscle fibres, their response can be dramatically different in various experimental conditions. Distinction of muscle satellite cell from myonuclei based on anatomical reference is possible using electron microscopy,[38]

TABLE 1 Changes in myonuclear content and apoptosis in response to various muscle atrophy models in rodents

Study	Specie	Muscle	Atrophy model	Atrophy duration	Muscle cross-section	Single muscle fibre	Muscle homogenate	Myonuclear content	Myonuclear apoptosis
Nuclear (ie Haematoxylin, TUNEL, Caspase, Bcl, Propidium idodide, Hoechst, DAPI, or Arcidine) staining only									
Tews et al[57]	Rat	Levator labii	Denervation	2, 6, 7, 10, 20 wk	x				Yes
Viguie et al[20]	Rat	EDL	Denervation	2, 4, 7, 12, 18 mon		x		Decline	
Yoshimura et al[43]*	Rat	Grac	Denervation	7, 14, 21, 28 d	x				Yes
Schmalbruch et al[22]*	Rat	EDL, Sol	Denervation	1, 2, 8, 22 wk	x			Decline	
Borisov et al[21]	Rat	TA, EDL, Sol	Denervation	2, 4, 7 mon	x				Yes
Jin et al[42]*	Rat	Brach. triceps	Denervation	16 wk	x				Yes
Wada et al[119]	Mouse	Plan	Denervation	4 mon		x		No change	
Aravamudan et al[19]	Rat	Dia	Denervation	2 wk		x		Decline	
Bruusgaard et al[34]	Mouse	EDL, Sol	Denervation/ mechanical unloading/ nerve impulse block	7, 14, 21 d		x		No change	
Lim et al[56]	Rat	Gas	Denervation	8 wk	x				Yes
Bruusgaard et al[15]*	Mouse	EDL	Denervation	14 d		x		No change	
Lee et al[54]*	Rat	Gas, Sol	Denervation	4 wk	x				Yes
Allen et al[25]	Cat	Sol	Spinal cord transection	6 mon		x		Decline	
Dupont-Versteegden et al[23]*	Rat	Sol	Spinal cord transection	10 d	x				Yes
Zhong et al[48]	Rat	Sol	Spinal cord transection	4, 60 d		x		No change	
Darr et al[26]	Rat	EDL, Sol	Mechanical unloading	3, 10 d	x	x		Decline	Yes
Kasper et al[45]	Rat	Gas, TA	Mechanical unloading	28 d		x		No change	
Allen et al[27]	Rat	Sol	Mechanical unloading	14 d		x		Decline	Yes
Allen et al[28]*	Rat	Sol	Mechanical unloading	14 d		x		Decline	Yes
Mozdziak et al[47]	Rat	Sol	Mechanical unloading	28 d		x		Decline	
Leeuwenburgh et al[29]*	Rat	Sol	Mechanical unloading	14 d	x				Yes
Dupont-Versteegden et al[51]*	Rat	Sol	Mechanical unloading	7 d	x				Yes
Jackson et al[44]*	Mouse	Sol, Gas	Mechanical unloading	14 d		x		No change	
Allen et al[31]	Rat	Sol	Space flight	14 d		x		Decline	
Kasper et al[46]	Rat	Gas, TA	Space flight	5.4 d		x		Increase	

(Continues)

TABLE 1 (Continued)

Study	Specie	Muscle	Atrophy model	Atrophy duration	Muscle cross-section	Single muscle fibre	Muscle homogenate	Myonuclear content	Myonuclear apoptosis
Lee et al[41]*	Rat	FHL	Detraining	20 wk		x		No change	
Dungan et al[40]*	Mouse	Plan	Detraining	12 wk		x		Decline	
Winje et al[36]*	Mouse	EDL, Sol, TA	Prostate cancer xenograft	6 wk		x		No change	NO
Nuclear (ie Dapi, Hoechst dye, TUNEL, EndoG) staining with cell border (ie Laminin or dystrophin) identification									
Bruusgaard et al[15]*	Mouse	EDL	Denervation	14 d	x			No change	No
Bruusgaard et al[34]*	Mouse	EDL, Sol	Denervation/ mechanical unloading/ nerve impulse block	7, 14, 21 d	x			No change	No
Dupont-Versteegden et al[23]*	Rat	Sol	Spinal cord transection	10 d	x			Decline	
Dupont-Versteegden et al[24]	Rat	Sol, Plan	Spinal cord transection	8 wk	x			Decline	
Allen et al[28]*	Rat	Sol	Mechanical unloading	14 d	x			Decline	Yes
Leeuwenburgh et al[29]*	Rat	Sol	Mechanical unloading	14 d	x			Decline	Yes
Hao et al[55]	Rat	Sol, Plan	Mechanical unloading	14 d	x				Yes
Bruusgaard et al[15]*	Rat	EDL, Sol	Mechanical unloading	14 d	x			No change	No
Jackson et al[44]*	Mouse	Sol, Gas	Mechanical unloading	14 d	x			No change	
Hikida et al[32]	Rat	Sol	Space flight	10 d	x			Decline	
Sandona et al[33]	Mouse	EDL, Sol	Space flight	20 d	x			Decline	
Egner et al[16]*	Mouse	EDL, Sol	Detraining	14 d, 3 mon	x			No change	No
Winje et al[36]*	Mouse	EDL, Sol, TA	Prostate cancer xenograft	6 wk	x			No change	No
Nuclear staining with cell border and satellite cell (ie Pax7 or NCAM) identification									
Adhihetty et al[39]*	Rat	TA, EDL	Denervation	5, 7, 14, 21, 42 d		x			Yes
Matsuba et al[30]	Mouse	Sol	Mechanical unloading	14 d		x		No change	
Lee et al[41]*	Rat	FHL	Detraining	20 wk		x		No change	
Dungan et al[40]*	Mouse	Plan	Detraining	12 wk		x		Decline	
Biochemical assay of muscle homogenate (ie RT-Pcr, Western blotting, Elisa)									
Yoshimura et al[43]*	Rat	Grac	Denervation	7, 14, 21, 28 d			x		Yes
Tang et al[53]	Rat	Gas	Denervation	30 d			x		Yes
Jin et al[42]*	Rat	Brach. tri	Denervation	16 wk			x		Yes
Alway et al[49]	Rat	Sol, Gas	Denervation	14 d			x		Yes

(Continues)

TABLE 1 (Continued)

Study	Specie	Muscle	Atrophy model	Atrophy duration	Muscle cross-section	Single muscle fibre	Muscle homogenate	Myonuclear content	Myonuclear apoptosis
Adhihetty et al[39]*	Rat	TA, EDL	Denervation	5, 7, 14, 21, 42 d			x		Yes
Lim et al[56]*	Rat	Gas	Denervation	8 wk			x		Yes
Lee et al[54]*	Rat	Gas, Sol	Denervation	4 wk			x		Yes
Alway et al[50]	Quail	Patagialis	Mechanical unloading	14 d			x		Yes
Siu et al[120]	RAT	Gas	Mechanical unloading	14 d			x		Yes
Dupont-Versteegden et al[51]*	Rat	Sol	Mechanical unloading	7 d			x		Yes
Hao et al[55]	Rat	Sol, Plan	Mechanical unloading	14 d			x		Yes
In vivo imaging									
Bruusgaard et al[34]*	Mouse	EDL, Sol	Denervation/Mechanical unloading/ nerve impulse block	7, 14, 21 d		x		No change	No
Bruusgaard et al[15]*	Mouse	EDL	Denervation	14 d		x		No change	no
Egner et al[16]*	Mouse	EDL, Sol	Detraining	14 d, 3 mon		x		No change	No
Winje et al[36]*	Mouse	EDL, Sol, TA	Prostate cancer xenograft	6 wk		x		No change	No

Note: This table provides an overview of studies that have assessed the change in myonuclear content and presences of (myo)nuclear apoptosis in response to various muscle atrophy models in animals. Studies are organized by analytical technique to assess (myo)nuclear content, apoptosis or both. EDL, extensor digitorum longus muscle; FHL, flexor hallucis longis; Sol, soleus muscle; Plan, plantaris muscle; TA, tibialis anterior muscle; Gas, gastrocnemius muscle; Grac, gracillis muscle.

*Note that these studies appear multiple times in the table, as multiple analyses were performed within the same study.

but remains very challenging with light or fluorescent microscopy. Hence, best practice would be to use a specific antibody that stains myonuclei only, and promising results have been shown using PCM1 labelling.[35] However, when nuclear labelling by for example DAPI or Hoechst is used, co-staining should be performed to delineate the cell border (eg laminin, dystrophin) and satellite cells (eg NCAM or Pax7) to make a reliable and valid estimation of myonuclear number. In the past, some [30,39-41] but certainly not all,[15-16,21,23-24,26,28-29,32-34,36,42-44] studies have used this approach to evaluate myonuclear content in response to different animal muscle atrophy models. Caution should also be taken when myonuclear number per fibre length is inferred from muscle cross-sectional data, as myonuclear shape and size have been observed to change in various experimental models.[22] Alternatively, myonuclear number can also be determined in mechanically isolated muscle fibres, which has the advantage that non-muscle nuclei are removed [15,19-20,25-28,31,34,36,40-41,44-48] (Table 1). Although fibre isolation allows inclusion of a relatively high number of myonuclei per fibre, the total number of evaluated fibres is often low, which may limit accurate representation of muscle tissue.

The loss of myonuclear content during atrophy appears to be in line with the frequently reported increase in the number of apoptotic (myo)nuclei in several animal studies[39,42-43,49-53] (Table 1). Apoptosis, or programmed cell death, is currently the (only) suggested mechanism through which myonuclei are removed from skeletal muscle tissue. Yet, evidence for myonuclear apoptosis during muscle atrophy remains rather ambiguous. For example, the increase in apoptotic markers (eg TUNEL, EndoG, Bcl, Caspase) observed following experimental animal atrophy models using biochemical assays (eg western blot, Elisa, RT-Pcr) is evaluated in muscle homogenates from which up to 50% of the nuclei are not myonuclei.[39,42-43,49-56] Most studies that reported an increase in the number of apoptotic nuclei on muscle cross-sections following atrophy were not able to accurately assess whether the nuclei were located in or outside the muscle fibre, due to the lack of a cell border markers.[21,23,26-28,42-43,54,56,57] When proper staining for the cell border is performed, only very low numbers of apoptotic (0.015%) myonuclei has been observed.[15,34,58] Nuclear destruction during apoptosis is, however, very rapid and comprises only 2-3 hours,[59] as such, some apoptotic activity in intact muscle fibres could not be entirely be ruled out. When apoptotic myonuclei are detected though, a small number of apoptotic myonuclei may likely reflect significant removal of myonuclei from skeletal muscle tissue. When nuclei are properly identified as myonuclei in muscle cross-sections (ie, nuclei located inside the muscle fibre), some do,[28-29,39,55] whereas others do not [15-16,34,36,58] report an increase in apoptotic myonuclei following muscle fibre atrophy in rodent muscle (Table 1). Yet, whether this discrepancy can be explained by the different markers used to identify apoptosis, the in/exclusion of satellite cells, and/or the model or duration of the induced atrophy remains to be further established.

2.1 | A potential paradigm shift

One of the first studies to challenge the supposedly established concept that myonuclei are lost during atrophy in animal skeletal muscle was performed by Bruusgaard et al[34] using direct in vivo time-lapse imaging of single muscle fibres. In this study, myonuclei were labelled with green-fluorescent protein (GFP) by somatic gene transfer using electroporation or intracellular injection. As the GFP label is based on water soluble oligonucleotides, and no gap junctions are present between satellite cells and the muscle fibre, muscle satellite cells are not labelled and, as such, automatically excluded from the analyses.[60] Using repeated exposure and imaging of the same fibre segments, changes in myonuclear content were assessed, in vivo, up to 4 weeks after initiating muscle atrophy. Although denervation, nerve blockage, or hindlimb suspension caused as much as 50% muscle fibre atrophy in adult mice, no changes in myonuclear content were observed.[34] Furthermore, the authors argued that nuclear apoptosis observed during atrophy was confined to muscle satellite and stromal cells, instead of myonuclei.[34] The ability to re-assess the same muscle fibre segments in vivo by time-lapse imaging may be the most accurate measurement to date to assess changes in myonuclear content during muscle fibre atrophy. However, it is important to note that the in vivo time-lapse imaging findings used in this study were in line with the change in myonuclear content determined by conventional histology (staining for dystrophin and Hoechst) on muscle cross-sections, the latter of which clearly contradicts earlier studies showing a loss in myonuclear content during atrophy using such a histological staining appro ach.[23-24,27,30,32-33,39,61] In line with these experimental muscle atrophy models, Schwartz et al[62] showed that myonuclear content also remains unchanged during naturally occurring hormonally triggered muscle atrophy (49% decrease in fibre CSA within 3 days) in the intersegmental muscle from the tobacco hawkmoth. The strength of this model is that the intersegmental muscle does not contain capillaries, satellite cells, endothelial cells or pericytes, meaning that all nuclei may be considered myonuclei.[63] However, the suggestion that the loss of 49% muscle mass within 3 days represents a model for the muscle mass loss during human aging over a period of 30 years would be too simplistic.[12] An alternative model of muscle fibre atrophy over a more prolonged period of time is cancer cachexia. Prostate cancer xenografting in mice has been reported to induce substantial muscle fibre atrophy (21%) over a 6-week period.[36] In line with the above findings, this study reported no changes in myonuclear

content assessed by in vivo imaging in single muscle fibres, as well as histological analyses of muscle cross sections.[36]

2.2 | Muscle memory by myonuclear permanence

The potential lack of myonuclear loss (by apoptosis) during extreme muscle atrophy models does not yet constitute convincing evidence for myonuclear permanence and the existence of muscle memory. The first experimental evidence for myonuclear retention following muscle hypertrophy was published by the Gundersen research group.[58] In this study, the extensor digitorum longus (EDL) muscle of mice was overloaded by synergistic ablation resulting in a significant increase in muscle fibre size and myonuclear content. More importantly, the elevated myonuclear number persisted during 3 months of subsequent denervation which was accompanied by severe muscle fibre atrophy.[58] Though a high degree of apoptosis was observed following 3 months of denervation, both long-standing as well as newly acquired myonuclei were excluded from this process.[58] Additional studies in mice demonstrated no changes in myonuclear content during 2 weeks of hindlimb suspension.[15,44] During 2 weeks of subsequent reloading, muscle fibre size was recovered back to baseline without any change in myonuclear content, suggesting that myonuclear addition may only occur in muscle fibres that undergo growth beyond their "baseline" size.[15,44] Further evidence supporting the muscle memory hypothesis was provided when the same group investigated the impact of anabolic steroid-induced muscle fibre growth in female mice.[16] Here, Egner et al[16] implanted pellets releasing testosterone propionate (or sham) subcutaneously for 14 days, with or without overload of the Soleus (Sol) and EDL muscle. A robust increase in muscle fibre size and myonuclear content were observed in response to the steroid treatment in both overloaded and non-overloaded muscles. One week after the steroid treatment was withdrawn, blood testosterone concentrations were back to baseline (undetectable levels). When the muscle was assessed three weeks later, myonuclear content remained 42% higher compared with the sham treatment group, whereas muscle fibre size had returned back to baseline levels.[16] When overload was subsequently introduced for a 14-day period, the group who had undergone previous steroid treatment showed a muscle fibre hypertrophy response which was more than double compared with sham-treated mice. Similar results were also observed when a 6-day overload period was introduced 3 months (constituting about ~12% of a mouse life span) after the testosterone propionate-releasing implant was removed in these mice.[16] Although muscle fibre hypertrophy was reversible in this model, a previous hypertrophic condition appeared to convey a lasting imprint on muscle fibres in the form of an elevated number of myonuclei, which contributed to the ability to regain muscle mass more quickly during a subsequent overload stimulus. In other words, a single episode of anabolic steroid use may have a long lasting, if not permanent, effect on the ability for muscle to (re)grow during training. This may pose a performance advantage for suspended doping offenders upon a return to elite competition and, as such, these findings led to several discussions related to the negative aspects of unfairness in sports. On a more positive note though, myonuclear permanence from prior training may, in theory, enhance treatment strategies to combat the loss of muscle mass later in life, whether it is associated with aging per se (i.e,. sarcopenia), or as a consequence of various clinical conditions. Importantly though, it still remains to be established whether age-related muscle fibre atrophy (obviously representing a process that takes place over a prolonged period of time) is accompanied by the concomitant loss of myonuclear content as this has been reported by some,[2,64,65] but not all [66,67] rodent studies. Overall, although more research is warranted to explain the apparent discrepancies within the literature, the existing evidence from animal studies definitely gives room for the possibility that myonuclei gained during muscle hypertrophic episodes earlier in life may at least partly be preserved to promote muscle tissue (re)growth later in life.

2.3 | Limitation of animal models

While the animal studies described above provide valuable biological insight, translating results to in vivo human settings remains a challenge. After all, as elegantly described by Demetrius, "*mice are not just small humans*".[18] For example, the time-frame in which the different animal models induce severe muscle atrophy (40%-50% loss in muscle tissue mass within 2-3 weeks) and/or hypertrophy (40%-50% gain in muscle tissue mass within 2-3 weeks) is something that does not occur in humans. As a reference, our research group has consistently demonstrated that quadriceps muscle cross-sectional area increases by merely 6%-10% following 12 weeks of progressive resistance exercise training.[10,68-72] Furthermore, the surgical procedure required to induce the atrophy/hypertrophy stimulus in rodents is stressful and can induce a robust immune response or cause degeneration/regeneration of muscle fibres. Finally, whereas overload induced by synergistic ablation in animals provides a persistent stimulus, exercise training in humans is characterized by relatively short episodes of anabolic stimuli interchanged with subsequent recovery periods. In an attempt to address some of these issues, a number of studies have been performed in animals that aimed to resemble a more physiological situation of exercise training as what would occur in the human situation. A study by Lee et al[41] used a rodent version of weighted "Jacobs ladder climbing" as an exercise modality to assess

the change in muscle mass/fibre size and myonuclear content (single fibre and muscle cross-sections) following 8 weeks of so-called "pre-training," followed by 20 weeks of detraining and 8 weeks of subsequent retraining. Pre-training resulted in a significant increase in *Flexor Hallucis Longis (FHL)* muscle mass/fibre size and myonuclear content.[41] Whereas muscle mass/fibre size was lost, myonuclear content remained unchanged during the subsequent detraining period.[41] During retraining, the increase in muscle mass/fibre size (+14.8%) was significantly greater compared with the pre-training period (+8.9%), with no further increase in myonuclear content,[41] much in line with myonuclear permanence paradigm. Contrasting results are, however, presented by Dungan et al[40] who exposed mice to 8 weeks of progressive weighted wheel running to induce muscle fibre hypertrophy, followed by 12 weeks of subsequent detraining. Here, the authors showed substantial (17%) muscle fibre hypertrophy and myonuclear accretion (~30%) in the *plantaris* muscle following the initial 8 weeks of exercise training. During the subsequent 12 weeks of detraining, both muscle fibre size and myonuclear content returned back to baseline levels.[40] Myonuclear content was assessed on both single muscle fibres as well as muscle cross-sections, in which muscle satellite cells were excluded from the myonuclear counts. Unfortunately, no final "retraining," nor any measurements of apoptosis were included in this study.[40] Whether these contradicting findings on the loss of myonuclear content during detraining can be explained by the model used to induce muscle fibre hypertrophy and myonuclear accretion, or any other study-related differences, remains to be further established.

Despite these discrepancies with regards to the muscle memory paradigm in a more physiological situation of muscle fibre hypertrophy and atrophy, the ultimate goal was to translate the observations on muscle memory to the in vivo human situation. As such, it is critical that these and other results are discussed in the light of observations made in experimental trials performed in humans.

3 | EVIDENCE FROM HUMAN STUDIES

The phenomenon that "skeletal muscle may hold some kind of memory" originates from observations in humans showing that previously trained individuals acquire muscle mass and strength more quickly upon retraining.[73-75] Staron et al[74] was the first to show that women regained their muscle strength and fibre size during 6 weeks of retraining as quickly as compared with the initial 20 weeks of strength training. Together with subsequent observations,[73-75] this has led to the suggestion that there may be some sort of local muscle memory responsible for such rapid muscle re-gain. However, it was much later before the first evidence from animal studies showing that myonuclei are not necessarily lost during atrophic conditions,[34] lead the authors to speculate on the "muscle memory by myonuclear permanence" hypothesis.[58] This hypothesis has since then been investigated and discussed almost exclusively based on studies performed in animals. Whether the postulated mechanisms also hold true in humans remains largely ambiguous. Nevertheless, there are several studies in humans that provide evidence to potentially support or contradict (parts of) the muscle memory hypothesis. For example, short-term (local) physical inactivity models in humans provide insight into whether myonuclei are lost during (acute) muscle fibre atrophy. Whereas some have reported no changes in myonuclear content when atrophy was induced by short-term single leg knee immobilization,[76-78] bed-rest,[79] or step reduction [80] protocols, others have shown that muscle fibre atrophy was accompanied by a small (5%-10%), yet significant, decline in myonuclear content following 14 days of bed rest[81] or micro-gravity exposure.[82] Previously, we have failed to detect any myonuclear content loss in critically ill patients despite severe muscle fibre atrophy (~20%) within as little as 7 days of being fully-sedated.[83] The general lack of consensus between studies may be explained in part by differences in the severity of the physical inactivity model applied (eg absoluteness, duration) resulting in differences in the observed muscle atrophy. Furthermore, myonuclear content in human studies is almost exclusively evaluated using muscle cross sections which may have, as discussed earlier, a limited ability to accurately detect small changes in myonuclear content over time. However, the studies performed in our laboratory [76-78,83] as well as others [80-82] appear to suggest that myonuclei are not lost in large amounts following muscle fibre atrophy induced by short-term physical inactivity.

3.1 | Muscle fibre size to myonuclear number ratio in humans

The myonuclear domain theory postulates that a linear relationship exists between muscle fibre size and myonuclear content, which has consistently been reported within human skeletal muscle tissue.[84-89] These findings are in contrast to animal data, which suggest that the relationship between muscle fibre size and myonuclear content is not consistent throughout the life span.[2] Over the past decade, we have evaluated type I and type II muscle fibre size and myonuclear content (excluding muscle satellite cells by Pax7 or NCAM co-staining) in over 400 untrained human volunteers with a wide age range from 18 to 89 years (Figure 1). A positive linear relationship exists between muscle fibre size and myonuclear content (Figure 1A and B). Interestingly, a similar positive association is also present between muscle fibre size and myonuclear domain size, is this case being expressed as a logarithmic correlation (Figure 1C and D). Post-hoc analysis

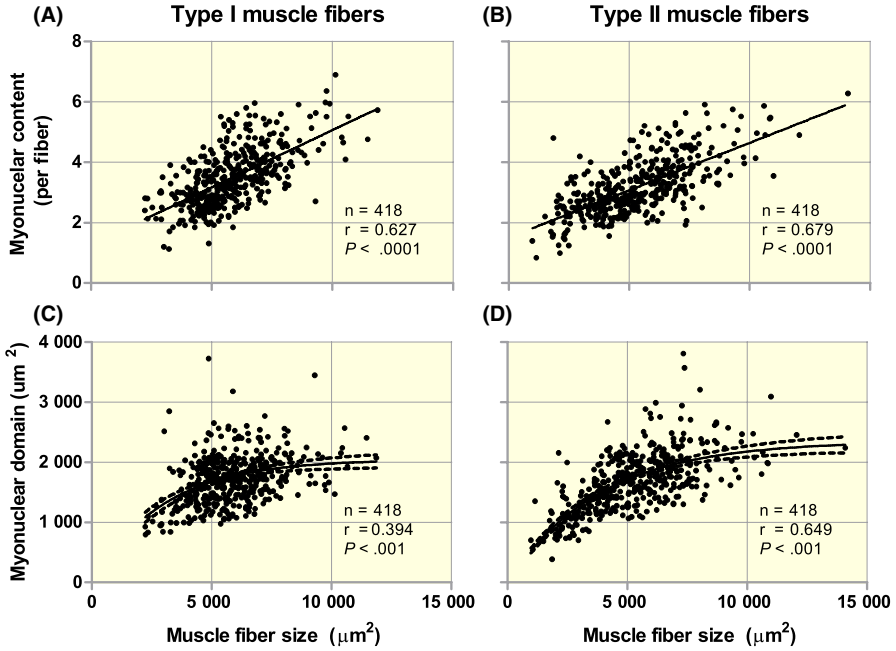

FIGURE 1 Correlation analysis between type I and type II muscle fibre size and number of myonuclei per fibre (A and B; linear relation) and myonuclear domain size (C and D; logarithmic relation) in percutaneous biopsy samples taken from the *vastus lateralis* of both healthy adult men (n = 330) and women (n = 88). All samples were collected with subjects at rest following an overnight fast. Muscle fibre size and myonuclear content were determined by immunofluorescent microscopy of muscle cross-sections. Staining included antibodies for laminin (cell border), MHCI (type I muscle fibres), Dapi (nuclei), Pax7 or NCAM (satellite cells). A Dapi + cell was considered to be a myonucleus when at least 50% of the staining was present within the muscle fibre identified by laminin staining. Muscle satellite cells were identified by Pax7 or NCAM staining and excluded from the myonuclear counts. At least 100 type I and 100 type II muscle fibres per subject were included to make a reliable estimation of myonuclear content

shows that the linear relationships between type I and type II muscle fibre size and myonuclear content are largely preserved with increasing age (see Figure S1A-D). As ageing is accompanied by a decline in muscle fibre size, which occurs predominately in type II muscle fibres, these data suggest that myonuclear content is flexible and unlikely to be retained indefinitely throughout the human lifespan. Most studies investigating muscle fibre characteristics are based on muscle biopsy samples obtained from male adults. Kramer et al[89] is one of the few studies performed in women, showing a positive association between muscle fibre size and myonuclear content in both healthy young (23 year) and older (82 year) women. Interestingly, the positive association was not observed in age-matched older (83 year) women suffering from a hip-fracture due to a low-energy fall (ie defined as falling from a standing height or less). Type II muscle fibre cross-sectional area was significantly smaller in the hip fracture patients as compared to age-matched healthy controls, but no differences were observed in myonuclear content. As a result, no relationship between myonuclear content and muscle fibre size was observed in the frail older women, who suffered a hip fracture.[89] Though these data are cross-sectional in nature, they may suggest that myonuclear content is retained to some degree when extensive muscle atrophy occurs in, in this case, frail older adults.

3.2 | Long-term myonuclear permanence in humans

Within the theoretical framework of muscle memory, it is postulated that myonuclei are maintained for an extensive period of time or maybe even indefinitely. This may subsequently represent a biological advantage when baseline muscle fibre size needs to be re-established after a period of disuse-induced muscle mass loss. Cross-sectional studies in humans provide additional evidence that potentially negates the proposition of indefinite myonuclear retention throughout life. For example, we have shown that type I and type II muscle fibre size as well as myonuclear content is substantially lower in severely atrophied muscle of spinal cord injured patients (~9 year after injury) compared with healthy age-matched controls.[90] Likewise, type II muscle fibre size and myonuclear content tends to be lower in patients suffering from multiple sclerosis compared with age-matched controls.[91] By far most studies in humans have focused on the comparison of relatively healthy young and older individuals. Whereas some studies showed a lower myonuclear content in old (>60 year) compared with young (18-29 year) participants,[89-90,92-94] others did not detect any differences,[9,87,95-97] (see Table 2). This discrepancy may be explained in part by the different age categories and/or the

TABLE 2 The effects of age on muscle fibre characteristics in humans

Study	Sex	Age, yrs (n)	Muscle fibre size	Myonuclear content	Myonuclear domain size
Cristea et al[121]	M[1]	21-32 (6) vs 72-96 (9)	I: ↑ (23%) IIa: ↓ (31%)	I: ↑ IIa: ↔	I: ↔ IIa: ↓ (33%)
	W[1]	24-32 (6) vs 65-96 (9)	I: ↑ (38%) IIa: ↓ (15%)	I: ↑ IIa: ↑	I: ↔ IIa: ↓ (41%)
Dreyer et al[95]	M[1]	21-35 (10) vs ≥ 60 (9)	I: ↔ II: ↓ (25%)	Mixed: ↔	
Hikida et al[122]	M[1]	23 ± 6 (7) vs 65 ± 6 (8)	Mixed: ↓ (31%)	Mixed: ↔	
Kadi et al[123]	M[2]	26 ± 3 (15) vs 74 ± 4 (13)		Mixed: ↑ (23%)	
	W[1]	23 ± 3 (16) vs 76 ± 3 (14)		Mixed: ↑ (23%)	
Kelly et al[124]	M/W[1]	26 ± 4 (27) vs 66 ± 4 (91)	I: ↔ II: ↓	I: ↔ II: ↔	I: ↔ II: ↓
Kramer et al[89]	W[1]	18-25 (15) vs ≥ 65 (15)	I: ↔ II: ↓ (30%)	I: ↔ II: ↓ (23%)	I: ↑ (31%) II: ↔
Mackey et al[113]	M[1]	24 ± 3 (12) vs 66 ± 4 (12)	I: ↔ II: ↔	I: ↔ II: ↔	I: ↔ II: ↔
Manta et al[125]	M/W[1]	17-30 (4) vs 31-60 (4)	Mixed: ↓	Mixed: ↓	Mixed: ↔
		17-30 (4) vs > 60 (7)	Mixed: ↓	Mixed: ↓	Mixed: ↑ (19%)
		31-60 (4) vs > 60 (7)	Mixed: ↔	Mixed: ↔	Mixed: ↑ (27%)
McKay et al[126]	M[1]	21 ± 3 (9) vs 70 ± 4 (9)	I: ↔ II: ↓ (21%)		I: ↔ II: ↓ (19%)
Petrella et al[9]	M[1]	20-35 (15) vs 60-75 (13)	Mixed: ↔	Mixed: ↔	Mixed: ↔
	W[1]	20-35 (16) vs 60-75 (14)	Mixed: ↔	Mixed: ↔	Mixed: ↔
Renault et al[127]	M/W[3]	23 ± 1 (6) vs 74 ± 4 (6)		Mixed: ↔	
Verdijk et al[96]	M[1]	20 ± 1 (8) vs 76 ± 1 (8)	I: ↔ II: ↓ (27%)	I: ↑ (17%) II: ↑ (13%)	I: ↓(16%) II: ↓ (31%)
Verdijk et al[90]	M[1]	31 ± 3 (8) vs 75 ± 2 (8)	I: ↔ II: ↓	I: ↔ II: ↓ (40%)	I: ↔ II: ↔
Verdijk et al[92]	M[1]	18-49 (50) vs 50-69 (53)	I: ↔ II: ↓ (18%)	I: ↔ II: ↔	I: ↔ II: ↔
		18-49 (50) vs ≥ 70 (49)	I: ↔ II: ↓ (29%)	I: ↔ II: ↓ (24%)	I: ↔ II: ↔
		50-69 (53) vs ≥ 70 (49)	I: ↔ II: ↔	I: ↓ (15%) II: ↓ (20%)	I: ↔ II: ↔
Verdijk et al[94]	M[1]	26 ± 2 (14) vs 72 ± 1 (16)	I: ↔ II: ↓ (26%)	I: ↓ (21%) II: ↓ (30%)	
Walker et al[128]	M[1]	27 ± 2 (5) vs 70 ± 2 (6)	I: ↔ II: ↓ (28%)	Mixed: ↔	Mixed: ↓
	W[1]	27 ± 2 (5) vs 70 ± 2 (5)	I: ↔ II: ↔	Mixed: ↔	

Note: Abbreviations: ↑, significantly higher compared with younger age category; ↓, significantly lower compared with younger age category; I, type I muscle fibres; II(a), type II(a) muscle fibres; M, men; M/W, men and women combined, ↔, no difference between age category; Mixed, mixed muscle fibre type; n, number of subjects included. Analyses were performed in the [1]vastus lateralis, [2]tibialis anterior or [3]biceps brachii/masseter muscle; W, women.

relatively low number of subjects included within some of the studies. Figure 2 shows the evaluation of type I and type II muscle fibre size, myonuclear content and myonuclear domain size in a large group (n = 302) of healthy men in different age categories. Here we show that type II muscle fibres are significantly smaller in older adults, which is accompanied by a lower myonuclear content as well as smaller myonuclear domain size (Figure 2). Again, although these data are cross-sectional, they do suggest that myonuclear content is not maintained indefinitely throughout the human lifespan. We have recently provided further evidence for this within a longitudinal exercise training

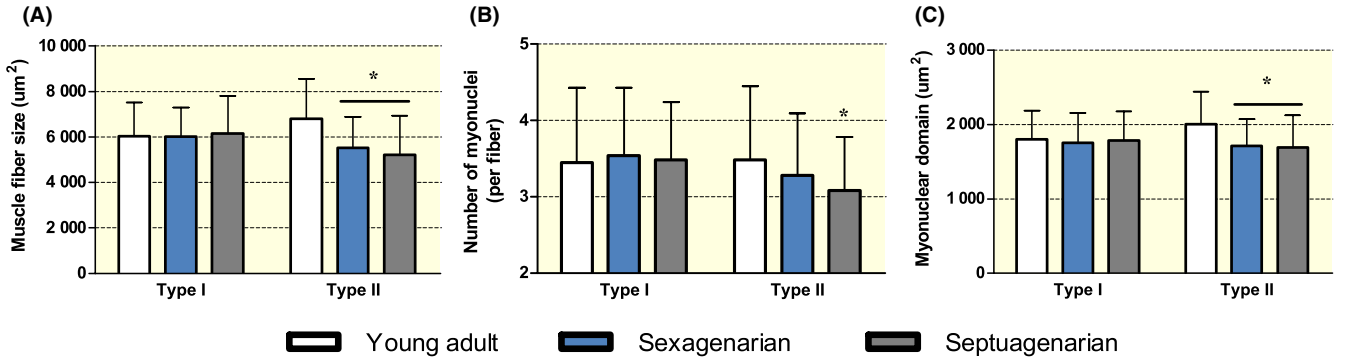

FIGURE 2 Type I and type II muscle fibre size (A), myonuclear content (B) and myonuclear domain size (C) in healthy young adults (18-29 y; n = 119), sexuagenarian (60-69 y; n = 91) and septuagenarian (70-79 y; n = 93). Data were analysed by means of a one-way ANOVA and are expressed as means ± SD. * Significantly different compared with young, $P < .01$. Horizontal line indicates that the differences are present in both groups

and detraining study.[98] In this study, healthy older adults (n = 35) were subjected to 6 months of supervised resistance exercise training, resulting in a significant increase in muscle fibre size and myonuclear content. Following 1 year of detraining, muscle fibre size as well as myonuclear content returned back to baseline levels.[98] These results appear to be in line with the earlier discussed study performed in animals, demonstrating a decline in myonuclear content during detraining.[40]

Overall, most studies in humans do not appear to support the idea that myonuclei are retained indefinitely throughout the lifespan. Yet, this does not necessarily negate the possibility that myonuclear domain size is flexible and/or that a relatively small myonuclear domain size (ie each myonuclei controls a smaller muscle fibre volume) may augment the muscle fibre (re)growth capacity. Figure 1 shows a large range in myonuclear domain sizes (from ~350 to ~3800 μm^2) measured in different individuals. Part of the muscle biopsy samples depicted in this figure actually constitute baseline samples of both young and older adults prior to performing a prolonged exercise training. This provides an opportunity to investigate whether myonuclear domain size at baseline may be a determining factor in the muscle fibre growth response induced by prolonged resistance exercise training. In a previous study, we showed a significant increase in muscle fibre size and myonuclear content in young men, which was not accompanied by changes in myonuclear domain size at any time point (2-4-8-12 weaks) during the 12-week resistance training program.[10] We concluded that large changes in myonuclear domain size are not apparent in a physiological situation when hypertrophy is induced by prolonged resistance exercise training.[10] We now re-analysed these data [10] and compared the hypertrophy response between individuals with a relatively small (<1700 μm^2, Small group (n = 9); mean ± SD myonuclear domain size of 1627 ± 91 μm^2) and large (>2000 μm^2, Large group (n = 13); mean ± SD myonuclear domain size of 2173 ± 223 μm^2) myonuclear domain size at baseline. Figure 3 shows muscle fibre hypertrophy following 2, 4, 8, and 12 weeks of resistance exercise training, with no differences between the Small and Large baseline myonuclear domain size groups. Interestingly though, myonuclear content increased earlier and to a greater extent in the group with large myonuclear domain size at baseline compared with the group with smaller myonuclear domain size at baseline. This is in line with the concept that (in humans) the myonuclear domain size may need to expand beyond a certain threshold before additional myonuclei are committed to the fibre.[99] Although myonuclear domain size was significantly different at baseline between the two groups, it is interesting to observe that following 12 weeks of exercise training, both groups end up with a myonuclear domain size around 1900 μm^2. These data are consistent with other studies performed in humans showing no relationship between baseline myonuclear domain size and muscle fibre hypertrophy response during exercise training.[99,100] Contrasting results are, however, observed when the same analytical approach is taken in one of our previous training studies in healthy older men.[70] In this study, muscle biopsies were only taken at baseline and following 12-weeks of progressive resistance exercise training. Interestingly, whereas a significant increase in muscle fibre size is observed in older men with a relatively small myonuclear domain size at baseline (<1600 μm^2 (n = 15); mean ± SD myonuclear domain size of 1431 ± 196 μm^2), no changes were observed in the Large group (>2000 μm^2 (n = 12) mean ± SD myonuclear domain size of 2046 ± 304 μm^2) following exercise training (Figure 4). Though we cannot determine the reason(s) why certain older adults have a smaller myonuclear domain size than others, these results may suggest that fibres with a small myonuclear domain size at baseline may undergo muscle fibre hypertrophy at a faster rate or greater extent during exercise training. Whether this effect is specific to older populations, who generally suffer from age-related type II muscle fibre atrophy, remains to be further established.

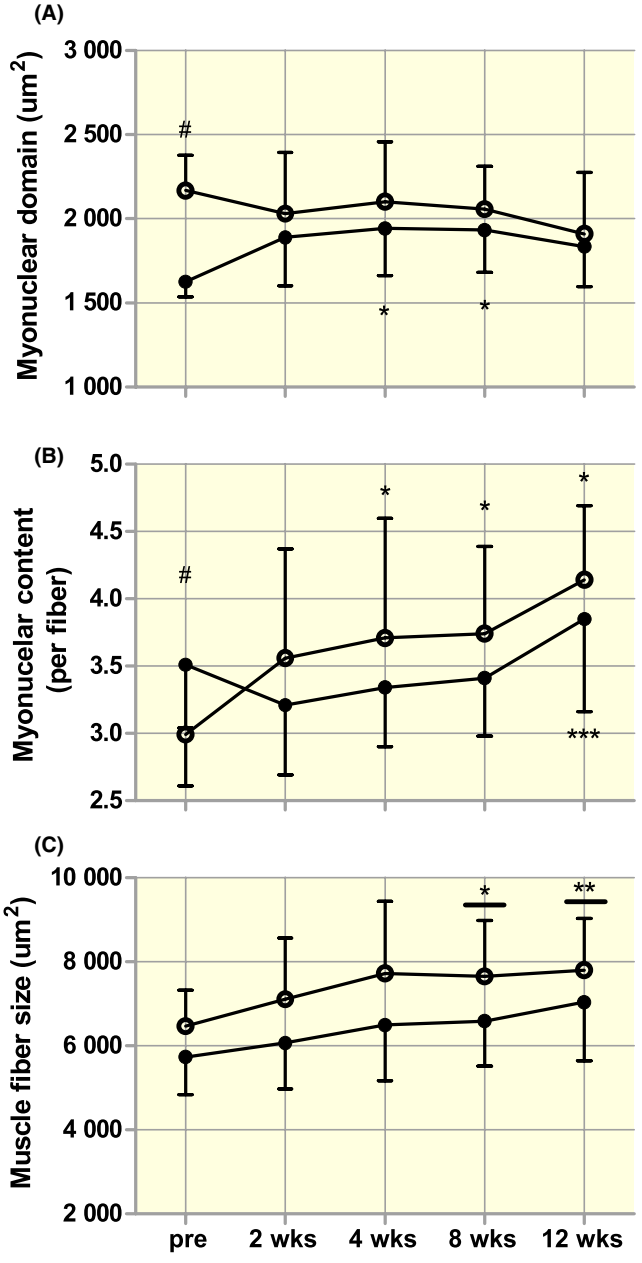

FIGURE 3 Changes in myonuclear domain size (A), myonuclear content (B) and muscle fibre size (C) in response to 2, 4, 8 and 12 weeks of progressive resistance exercise training in healthy young men with a relatively small (<1700 μm^2; Small group; n = 13) or large (>2000 μm^2; Large group Large group; n = 10) myonuclear domain size at baseline. Myonuclear content and fibre size were determined by immunofluorescent microscopy as described previously.[10] Muscle satellite cells were identified by NCAM staining and excluded from the myonuclear counts. Data were analysed with a two-way repeated measures ANOVA with time (pre, 2, 4, 8 and 12 weeks) as within subject factor and group (Small vs Large) as between subject factor. A significant group × time interaction was observed for myonuclear content ($P < .05$) as well as myonuclear domain ($P < .05$), resulting in separate one-way repeated measures ANOVAs and pairwise comparisons being performed to identify within-group effects. Data are expressed as means ± SD. *Significantly different compared with pre, $P < .05$. **Significantly different compared with pre and 2 weeks, $P < .05$. *** Significantly different compared with 2 and 4 weeks, $P < .05$. # Significantly different between groups pre, $P < .05$. Horizontal line indicates that the effect is present for both groups

Of note, comparing the physiological response of exercise training based on "extreme" baseline characteristics can provide insight into potential mechanisms, but care should be taken when extrapolating these kind of observations. As discussed earlier, determining myonuclear content (and as such myonuclear domain size) in human muscle cross-section is not without uncertainty. Furthermore, comparing individuals based on "small" vs "large" values at baseline opens up the possibility of statistical regression towards the mean, which is a concern that should be taken into consideration when interpreting the observations made. Nonetheless, the human data currently available appear to indicate that although some myonuclei may be lost throughout life, a low myonuclear domain size (whether or not the result of myonuclear permanence) may beneficially affect the muscle fibre (re)growth capacity.

3.3 | Testing muscle memory by myonuclear permanence in humans

Currently there is only one study that attempted to directly test the hypothesis of muscle memory by myonuclear permanence in humans. In the study by Psilander et al,[11] healthy young men performed 10 weeks of unilateral resistance exercise training, followed by 20 weeks of detraining and a subsequent 5 weeks of bilateral retraining. Skeletal muscle thickness and strength increased significantly in response to the initial exercise training period, which was mostly lost during the successive 20 weeks of detraining. During retraining, however, no differences were observed in muscle thickness or strength gains between the previously trained leg and the untrained leg. Muscle biopsy samples were collected before and after the initial 10-week training period and from both legs prior to and following 5 weeks of retraining. In line with the muscle thickness and strength data, both type I and type II muscle fibre size increased significantly in response to the initial 10 weeks of exercise training (+13 and + 17% respectively). However, muscle fibre size remained unchanged during detraining as well as 5 weeks of bilateral retraining. Furthermore, no changes in myonuclear content could be detected in response to the initial training, detraining, or retraining period.[11] The degree of muscle fibre growth observed during the initial 10 weeks of exercise training may have been too small to elicit a group mean increase in myonuclear content which is required to firmly establish whether muscle memory by myonuclear retention exists in humans. As Psilander et al[11] published their raw study data, a secondary analysis on these data was recently performed by Murach and colleagues. Here, Murach et al[12] retrospectively

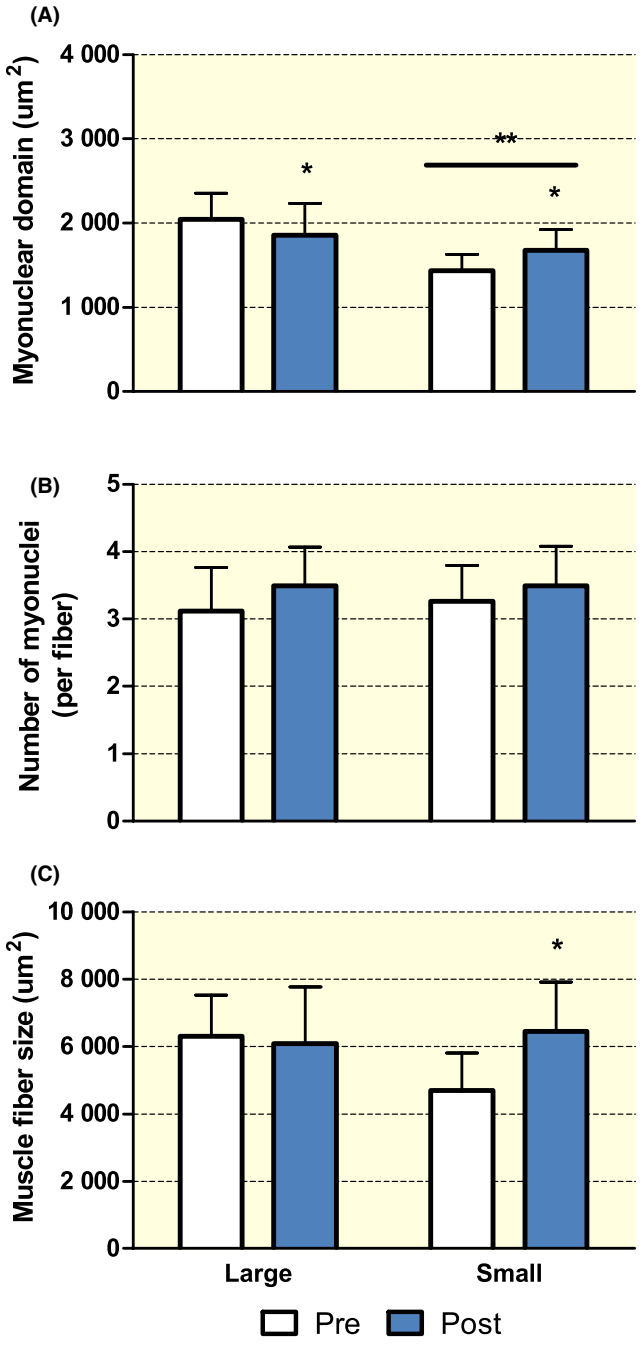

FIGURE 4 Changes in myonuclear domain size (A), myonuclear content (B) and muscle fibre size (C) in healthy older adults with a relatively small (<1600 μm^2; Small group; n = 15) or large (>1800 μm^2; Large group; n = 12) myonuclear domain size at baseline (pre) and after (post) 12 weeks of progressive resistance exercise training. Myonuclear content and fibre size were determined by immunofluorescent microscopy as described previously[10]. Muscle satellite cells were identified by NCAM staining and excluded from the myonuclear counts. Data were analysed with a two-way repeated measures ANOVA with time (Pre vs Post) as within subject factor and group (Small vs Large) as between subject factor. A significant group × time interaction was observed for muscle fibre size ($P < .05$) as well as myonuclear domain size ($P < .05$), resulting in separate one-way repeated measures ANOVAs and pairwise comparisons being performed to identify within-group effects. Data are expressed as means ± SD. *Significantly different compared with Pre, $P < .05$. **Significantly different compared with Large group, $P < .05$. Horizontal line indicates that the effect is present for both time points

divided the participants in two groups, based on the immunohistochemical analyses of both muscle cross sections and single muscle fibres: one group in which a significant increase in myonuclear content was observed (n = 11) following the initial training program, and one group in which no change was observed (n = 8). Since nine out of the eleven subjects who demonstrated an exercise induced increase in myonuclear content, subsequently showed a decline during detraining, the authors suggested that the "newly acquired myonuclei" during exercise training are not being retained during detraining.[12] However, the findings might easily also be explained by a regression towards the mean, without any underlying loss of myonuclei as was also rightfully addressed by the authors of the original paper in their recent letter-to-the-editor[13] as a response to this "secondary analysis." Based on this, as well as the other discussed studies, it is safe to say that there is currently no consensus within the scientific community on the existence of muscle memory by myonuclear permanence in human skeletal muscle and more research is warranted using properly designed intervention studies.

3.4 | Muscle fibre size cluster approach

Data presented here, as well as in most other papers, report the mean in muscle fibre size, myonuclear content and domain size of a muscle biopsy sample. However, it has previously been shown that grouping muscle fibres of similar sizes into clusters may provide a more detailed insight in the relationship between muscle fibre size and myonuclear content during muscle fibre growth.[64,87,93] The application of this approach has already shown that the smallest muscle fibres (<3000 μm^2) have a disproportionally small myonuclear domain size compared with larger (3000-7000 μm^2) muscle fibres in humans.[87,93] Hence, it has been speculated that smaller muscle fibres with a (disproportionally) small myonuclear domain may have a greater muscle fibre hypertrophy potential in response to resistance exercise training when compared to larger muscle fibres. This may be of particular relevance in older adults who have a relative high number of smaller muscle fibres due to age-related muscle fibre atrophy. However, thus far only one study has reported on the myocellular changes in different muscle fibre size clusters in response to exercise training. Karlsen et al[93] evaluated muscle tissue samples taken from very old older adults (83-94 year) who performed 12 weeks of resistance exercise training. Although the exercise training program resulted in a significant increase in muscle strength, no changes in muscle

fibre size, myonuclear content and/or domain size were observed in the muscle biopsy samples.[93] The lack of muscle fibre hypertrophy limited the authors' ability to draw any firm conclusions on whether the smaller muscle fibres (with a disproportionately small myonuclear domain size) may have a larger muscle fibre growth potential in response to exercise training. Most human studies, including the discussed studies of Karlsen et al,[87,93] evaluate myonuclear content using muscle cross-sections which is, as discussed earlier in this review, not without uncertainty. However, analysing myonuclear content in a large number of cross-sectional muscle fibres does allow to account for the apparent heterogeneity in myonuclear to muscle fibre ratio observed in different muscle fibre size clusters. In contrast, though in vivo imaging techniques or single fibre isolations may provide a more accurate assessment of myonuclear content per se, these techniques are limited by the inclusion of only "a few surface fibres" or a relatively low number of fibres, respectively, which hampers the ability to account for fibre heterogeneity.[34] Obviously, such pro's and con's should be taken into consideration when evaluating data using these different techniques. Clearly more research is warranted, but the muscle fibre cluster approach may be a valuable tool to provide more insight in the role of myonuclei during muscle reconditioning, and as such, in the potential existence of muscle memory by myonuclear permanence.

4 | FUTURE RESEARCH DIRECTIONS

Further experimentation is clearly warranted to more firmly establish whether muscle memory by myonuclear retention exists and, more importantly, whether it plays a relevant role in muscle reconditioning in humans. The most effective study designs should be comparable to that of the recently published study by Psilander and colleagues,[11] who applied a unilateral approach. Moreover it is critical that exercise training intensity, volume and/or duration progresses enough to induce considerable (and measurable) muscle fibre hypertrophy as well as myonuclear accretion. For example, 12 weeks of lower-body training, three times weekly with weight progression re-assessed weekly has consistently been shown to induce an ~25% increase in muscle fibre CSA in younger and older participants.[9-10,70,104-106] Such an exercise training period should then be followed by a detraining period of at least equivalent duration, allowing muscle fibre size to return almost entirely to baseline values (with or without a decline in myonuclear content). Subsequently, volunteers should then undergo retraining in both the prior-trained and untrained leg separately to assess whether the increase in muscle fibre size (as well as muscle mass and strength) is more pronounced in the prior-trained leg when compared with the initial training programme response, as well as when compared with the increase observed in the previously untrained leg.

4.1 | Methodological considerations

To further advance this field of research, the accurate assessment of myonuclear content in muscle tissue is also of critical importance. Since in vivo time lapse imaging of myonuclear content is limited to animal models, determining myonuclear content in humans will most likely rely on histological analyses of muscle cross sections and/or single muscle fibres. It is essential to differentiate between nuclei that are located in- and outside the muscle fibres, and it is best practice to exclude muscle satellite cells from the myonuclear counts. As such, a staining approach should be used that combines a nuclear stain (eg DAPI or Hoechst dye) with specific antibodies that delineate the muscle cell border (eg laminin or dystrophin antibody) and identify muscle satellite cells (eg NCAM or Pax7 antibody). The validation of myonuclear specific antibodies, like PCM1,[35] will most likely also provide a substantial improvement in the reliability and sensitivity of myonuclear content measurement required to accurately assess changes in response to muscle fibre hypertrophy and atrophy models. As myonuclear content has been shown to differ between smaller and larger muscle fibres within single muscle cross-sections, detailed muscle fibre size cluster analyses on myonuclear content and domain size may also provide further insight in the regulation of myonuclear content during muscle fibre reconditioning. Finally, more research is required (especially in humans) that can establish through which mechanism(s) myonuclei are potentially lost (if at all) under conditions of muscle fibre atrophy. Although myonuclear apoptosis has been suggested for many years as the mechanisms for the removal of myonuclei from intact muscle fibres, the evidence for this remains rather circumstantial. It will remain critical to determine whether the nuclear apoptosis typically observed during muscle atrophy represents true myonuclei or other cell types like stromal and satellite cells.

4.2 | Measuring DNA synthesis rates

While histological imaging is applied to assess static changes in myonuclear content between time points, alternative methods have been applied recently to provide dynamic insight in myonuclear regulation. The process of myonuclear addition to a muscle fibre requires satellite cell activation, replication and differentiation, during which DNA synthesis occurs.[11] Stable isotope tracer approaches have been successfully applied to label nucleotide base pairs (ie, pyridine deoxyribonucleotides) and measure DNA synthesis rates in the blood and muscle tissue of rodents and humans.[17-111] Robinson et al[18]

were the first to orally administer deuterated water (^2H_2O) and assess muscle DNA synthesis rates over several weeks in humans. The investigators reported that DNA synthesis rates were greater in a group of older males during 6 weeks of high-intensity endurance training (0.15%/d) when compared with a group of young males who remained at rest. The elevated DNA synthesis rates suggest that new myonuclei are synthesized in muscle during exercise training, which aligns with several studies demonstrating an increase in myonuclear content over prolonged exercise training.[9-10,98-99,104,112-115] In the young, rested males, DNA synthesis rates were not statistically greater than zero, meaning that DNA synthesis was undetectable.[18] This finding is perhaps relevant to the myonuclear permanence hypothesis as it seems to provide some indication that myonuclear synthesis is either dormant or too low to detect in (untrained) humans under resting conditions. These findings may explain why myonuclear content remains relatively stable until older age and senescence.[92] Whether or not the training-induced elevation in DNA synthesis rate returns to "dormancy" during a period of detraining and then remains dormant during re-training (which would be supportive of the muscle memory paradigm) remains to be established. Such data, coupled with histological myonuclear content and cellular apoptosis measurements, would provide better insight into the underlying regulation of myonuclear content and add to our understanding of myonuclear permanency and the muscle memory hypothesis. It is important to note that DNA isolation from muscle currently represents a mixed DNA pool, derived not only from satellite cells, but also from innate immune cell infiltration and fibroblast activity among other muscle-borne cell types. While Robinson et al.[18] argue that the DNA synthesis measurement primarily represents DNA synthesis derived from the satellite cell pool, future work should also aim to further refine the DNA extraction protocol in muscle tissue to specifically isolate the satellite cell and/or myonuclear pools (and note that fractional synthetic rate is only part of the balance between synthesis and repair, and never predicts absolute hypertrophy or in this case net myonuclear accretion).

4.3 | Epigenetic muscle memory

Other than the existence of muscle memory by myonuclear retention, it has been hypothesized that muscle memory may also find its origin at the epigenetic level. Seaborne et al[75] demonstrated that 7 weeks of resistance exercise training-induced muscle fibre hypertrophy was accompanied by DNA hypomethylation. Whereas muscle mass returned back to baseline during subsequent detraining, DNA hypomethylation was maintained. Moreover, this hypomethylation was also maintained during a succeeding 7-week period of resistance exercise re-training, during which muscle mass and strength gains were significantly greater as compared with the initial 7-week period of exercise training.[75] Hypomethylation generally promotes enhanced gene expression due to the removal of methyl groups from the DNA, allowing improved access of the transcriptional machinery and RNA polymerases that enable transcription. Together this would suggest that exercise training leads to an enhanced hypomethylated state of genes, which is then maintained during detraining, enabling greater transcription of these genes during retraining and, as such, allowing an enhanced muscle fibre growth response. It remains to be determined whether or not training-induced DNA hypomethylation persists over a more prolonged period, independent of changes in myonuclear content, and promotes greater muscle fibre hypertrophy during retraining after a longer period of detraining (even up to decades).

5 | EVOLUTION VS TELEOLOGY

It has been speculated that muscle memory by myonuclear permanence may have an evolutionary origin.[37] Permanent retention of myonuclei might represent an adaptive mechanism to individuals who had the need for increased muscle performance (ie strength) in the past to regain muscle mass faster in the future, as a more long-term advantage or adaptation. Hence, during less labour intensive time periods, maintenance of a large amount of muscle mass can be avoided, but the capacity to regain that muscle mass when necessary is maintained. In other words, the experience from the past becomes useful when the same task arises again, similar as observed with memory of the brain. However, since there are quite a number of studies that currently appear to contradict the myonuclear permanence hypothesis (as discussed within the review) and it also poses several challenges to conventional scientific thought of the "use it or lose it" principle, as such, it is also worth considering a more teleological perspective. As a rule, efficiency is an underpinning of basic biology. Cells/tissues/organs have an ability to adapt to stress by adding organelles or even anatomical structures to support demand. Classic examples include the addition of mitochondria and capillaries following aerobic training.[116,117] These are essential adaptations designed to promote greater oxygen delivery and optimize substrate use during exercise. Importantly, however, mitochondria and capillaries are rapidly lost when exercise training is not continued.[118] These are two classic examples of biological efficiency in the context of exercise science. Therefore, it is important to ask the question: does it make sense that a muscle cell would keep myonuclei when they are not needed? This is a particularly relevant question when one considers that muscle cells have a readily available source of nuclei (by the means of satellite cells) that can be rapidly mobilized following a stressful event like the early

stages of an exercise training program. Although the teleological perspective may be equally thought-provoking, it also largely remains speculation. Particularly, the costs of keeping a high number of myonuclei in a small muscle fibre has never been quantified, nor compared with the cost of eliminating and recreating myonuclei during muscle adaptation. Hence, despite the fact that new myonuclei can be generated, it may be equally "efficient" to simply maintain previously generated myonuclei, which would then be readily available for future (re)use.

The epigenetic hypothesis of muscle memory may, however, be an alternative plausible mechanism to consider. Indeed, epigenetics, the modification of genes to enhance or repress gene expression, may proof to be a credible mechanism to explain the concept of muscle memory. Furthermore, muscle memory by myonuclear permanence and epigenetics are also not mutually exclusive. Ultimately, it will be the collection of definitive data using appropriate models that will shed light on this issue. At present, though it has potential as a theory, muscle memory by myonuclear permanence fails to pass (at least in humans) the litmus test at this stage**.**

6 | CONCLUSION

The current scientific evidence provides no consensus on the existence of muscle memory by myonuclear permanence in both animal or human skeletal muscle tissue. Clearly, more research is warranted, particularly in humans, to experimentally test the muscle memory hypothesis. Developing more sensitive methods to evaluate changes in myonuclear content and function in muscle biopsy samples may be required to fully establish the intricate role that myonuclei play in muscle reconditioning.

CONFLICT OF INTEREST

None of the manuscript authors have any conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Snijders T, Aussieker T, Holwerda A, Parise G, van Loon LJC, Verdijk LB. The concept of skeletal muscle memory: Evidence from animal and human studies. *Acta Physiol*. 2020;229:e13465. https://doi.org/10.1111/apha.13465