

REVIEW

Muscle memory and a new cellular model for muscle atrophy and hypertrophy

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ABSTRACT

Memory is a process in which information is encoded, stored, and retrieved. For vertebrates, the modern view has been that it occurs only in the brain. This review describes a cellular memory in skeletal muscle in which hypertrophy is ‘remembered’ such that a fibre that has previously been large, but subsequently lost its mass, can regain mass faster than naive fibres. A new cell biological model based on the literature, with the most reliable methods for identifying myonuclei, can explain this phenomenon. According to this model, previously untrained fibres recruit myonuclei from activated satellite cells before hypertrophic growth. Even if subsequently subjected to grave atrophy, the higher number of myonuclei is retained, and the myonuclei seem to be protected against the elevated apoptotic activity observed in atrophying muscle tissue. Fibres that have acquired a higher number of myonuclei grow faster when subjected to overload exercise, thus the nuclei represent a functionally important ‘memory’ of previous strength. This memory might be very long lasting in humans, as myonuclei are stable for at least 15 years and might even be permanent. However, myonuclei are harder to recruit in the elderly, and if the long-lasting muscle memory also exists in humans, one should consider early strength training as a public health advice. In addition, myonuclei are recruited during steroid use and encode a muscle memory, at least in rodents. Thus, extending the exclusion time for doping offenders should be considered.

KEY WORDS: Skeletal muscle, Muscle memory, Atrophy, Hypertrophy, Satellite cells, Myonuclei, Strength training

Introduction

A memory process entails encoding, storing and retrieving information. For vertebrates, the modern view has been that this process only occurs in the brain, where it is probably related to long-lasting changes in synaptic efficiency. There has been a notion that other organs or the body as a whole might also have a form of ‘body memory’. For example, in popular culture it has been envisioned that transplanted organs might confer some form of personality transfer.

The concept has also been used in more serious psychological literature on child abuse, where the bodies of the victims supposedly remember the traumatic incidence (Koch et al., 2012). It has been unclear whether the term is used literally or metaphorically, but in any case, the notion has been largely discredited and body memory has been considered to be a pseudoscientific concept (Smith, 1993).

More credible is the use of the term memory in the immune system to describe the fact that on the second encounter of an antigen, the immune response is stronger and more rapid. This memory is stored in memory immune cells; for example, memory T

cells exposed to a specific antigen have a different molecular makeup compared with naive T cells (Mackay, 1999).

The term cell memory has also been used to describe irreversible programming of cells such as stem cells, and is attributed to epigenetic mechanisms such as histone and DNA modifications other than changes in base sequence (e.g. Alvarez and Margulies, 2014; Li and Zhang, 2014). For example, it has been demonstrated that fibroblasts in culture maintain a specific gene expression pattern depending on where in the body they are taken from, and this has been dubbed a positional memory (Chang et al., 2002). The more traditional term for such phenomena is cell differentiation. Although some forms of cell differentiation might fill the formal criteria for memory as defined in the first paragraph above, perhaps the term should be reserved for latent, lasting, adaptive changes that are caused by environmental factors.

In relation to skeletal muscle, René Descartes (1596–1650) wrote: ‘The lute players have part of their memory in their hands, because the facility to move and bend their fingers in various ways which they have acquired by habit, helps them to remember passages that require them to move their fingers in a way in order to play them’ (Koch et al., 2012). In this statement, Descartes seems to believe that there is a memory in the fingers themselves, and such ideas have probably led to the term muscle memory. Used this way, it is an unfortunate term, because our current understanding is that learning to play the lute and similar tasks is a form of motor learning in the central nervous system (CNS), and not the muscles. Thus, what has scientifically been called muscle memory so far is really synonymous to motor learning. Motor learning has been treated in a recent review (Diedrichsen and Kornysheva, 2015) and is outside the scope of this paper.

The term muscle memory has also been used for the ability to rebuild muscle mass and strength, as previous strength exercise seems to make it easier to regain muscle mass later in life even after long intervening periods of inactivity and mass loss (Staron et al., 1991; Taaffe and Marcus, 1997). This phenomenon has hereto, however, also been solely attributed to motor learning in the CNS (Rutherford and Jones, 1986). There is clearly a CNS component to the muscle memory of strength training, as the increase in force may precede the increase in muscle mass, possibly by altering spinal motor neuron excitability, and induce synaptogenesis within the spinal cord (Adkins et al., 2006). For strength, however, the build-up of muscle protein is crucial, and a purely neural mechanism has not seemed satisfactory.

Results from recent experiments suggest that there is a form of cellular memory in the muscle cells themselves explaining the phenomenon that muscle mass previously obtained is easily regained, and that the cellular mechanism for this ‘memory’ is related to the number of myonuclei (Bruusgaard et al., 2010; Egner et al., 2013; Gundersen, 2011).

The largest cells of the body

Muscle cells are peculiar in being by far the largest cells of the mammalian body. In mice, limb muscle fibres have a typical volume

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of 5 nl (Utvik et al., 1999). In larger mammals, volumes can be much higher; e.g. a cylinder with a diameter of 50 μm and a length of 0.5 m has a volume of $\sim 10^3$ nl (Bruusgaard et al., 2003). Most other mammalian cells are more spherical, with diameters in the range of 5–20 μm and volumes ranging from 10^{-5} to 10^{-3} nl, i.e. many orders of magnitude smaller than a muscle fibre. The muscle cells have multiple nuclei, however, and constitute one of the few syncytia in the mammalian body.

It has been suggested since the 19th century that a nucleus can serve only a certain volume of cytoplasm (e.g. Strassburger, 1893), and it was recently argued that the link between DNA content and cell volume is a fundamental principle in biology (Gregory, 2001). In muscle it is believed that each nucleus serves a certain domain, and it has been shown that some proteins are localized to the site of expression both *in vitro* (Hall and Ralston, 1989; Pavlath et al., 1989; Ralston and Hall, 1992) and *in vivo* (Gundersen et al., 1993; Merlie and Sanes, 1985; Sanes et al., 1991). Each nucleus is surrounded by a synthetic machinery that seems to remain localized (Pavlath et al., 1989).

In its strictest version, this nuclear domain theory has implied that a nucleus supports a constant volume of cytoplasm. We have studied the naturally occurring range of fibre sizes of limb muscles of the mouse using *in vivo* imaging. In the slow/oxidative soleus muscle of young adult animals (2 months), the strict nuclear domain theory was largely confirmed as the number of nuclei was reasonably proportional to the cytoplasmic volume, but in the fast/glycolytic extensor digitorum longus, the number of nuclei varied proportional to fibre surface, and thus large fibres had systematically larger cytoplasmic volume domains than smaller fibres (Bruusgaard et al., 2003). Similarly, various degrees of correlation between fibre size and number of myonuclei have been found in fixed and isolated fibres from young animals (Brack et al., 2005; Mantilla et al., 2008; Wada et al., 2003).

Notably, the common observation that there is at least some correlation between size and number of nuclei is not universal. Thus, in older mice (14–18 months) there was no correlation between fibre size and the number of nuclei, in spite of a fourfold variability in size (Bruusgaard et al., 2006; Wada et al., 2003). We speculate that the number of nuclei displayed in these older mice reflects not only the acute size of the fibre, but also its history, as a form of memory.

The syncytial nature of muscle fibres might be related to the lack of a long-distance transport system for proteins within these large cells. Thus, the myonuclei are optimally positioned as to minimize transport distances within each fibre (Bruusgaard et al., 2003, 2006), and perturbation in the positioning of nuclei leads to impaired muscle function (Metzger et al., 2012).

The syncytial nature and the correlation between nucleic number and cell volume, observed at least under some circumstances, suggest that there is a rate-limiting step related to each nucleus' capacity for protein synthesis. In particular, during hypertrophic growth it does not seem plausible that, for example, one nucleus could provide all the RNA required to support the vast cytoplasmic volume of muscle fibres, but the nature of the bottleneck is not known.

Interestingly, it was recently suggested that ribosome biogenesis plays a central role during muscle hypertrophy (Chaillou et al., 2014). For example, the 47S pre rRNA transcribed by Pol I is increased during overload hypertrophy (von Walden et al., 2012). rRNA accounts for 70% of all transcription, and most steps in the assembly of the ribosomal subunits take place in the nucleolus, where rRNAs are transcribed as large precursors, which undergo extensive nucleotide modification. In addition, more than 200 non-ribosomal, resident nucleolar proteins are required to process

and modify the rRNAs and to aid their assembly with the ~ 80 ribosomal proteins (Granneman and Tollervey, 2007). These processes require efficient trafficking across the nuclear membrane. In growing yeast, each nuclear pore is believed to import 1000 ribosomal protein molecules per minute, and export ~ 25 ribosomal subunits per minute (Warner, 1999). Although the numbers are probably less dramatic in mammalian cells, such high-throughput processes in the nucleus provide many good candidates for steps that might be limiting hypertrophic growth and act as a bottleneck if the number of nuclei is not sufficiently high.

The textbook model for muscle size regulation

Changes in muscle fibre size are accomplished by altering the balance between protein synthesis and proteolysis (Fig. 1). The molecular signalling mechanisms regulating these processes have been the subject of several recent reviews (e.g. Cohen et al., 2015; Egerman and Glass, 2014; Gundersen, 2011; Schiaffino et al., 2013), and the focus has mainly been on regulating protein degradation and synthesis per nucleus.

A corollary to the idea of constant myonuclear domains is that the number of nuclei should change in proportion to size during atrophy and hypertrophy. This is reflected in the current textbook model for the regulation of muscle mass, which is illustrated in Fig. 2. The model implies that during hypertrophic growth, myonuclei are recruited from muscle stem cells residing inside the fibre basal lamina (hence called satellite cells), resulting in a large fibre with many myonuclei. The higher number of nuclei should contribute to the increase in protein synthesis because total protein synthesis is the product of synthesis per nucleus and the number of nuclei (Fig. 1).

During atrophy, the model suggests that myonuclei are eliminated by apoptosis (Fig. 2). This would have to be a selective nuclear apoptosis of some of the nuclei within the intact muscle fibre. Notably, according to this model, a fibre undergoing hypertrophic growth, e.g. during strength exercise, and then returning to initial size will appear identical to a fibre that had never undergone growth. It ends up where it started, as a small fibre with few nuclei. There is no apparent hysteresis or memory according to this model. However, both the ascending and the descending limbs of the model have now become controversial.

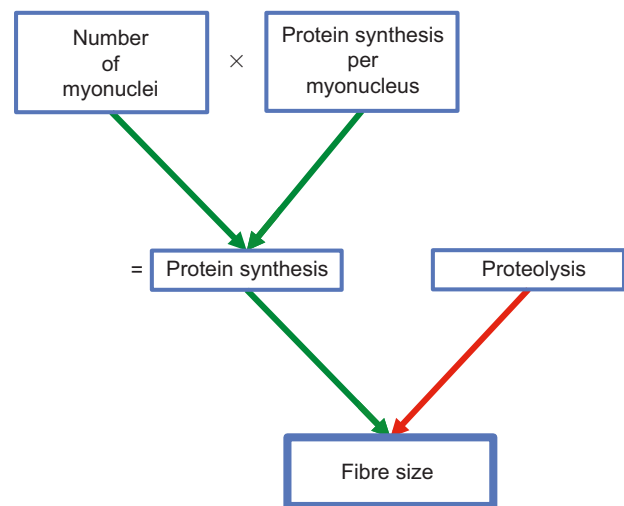


Fig. 1. Major determinants of muscle fibre size. Changes in fibre size occur by changing the balance between protein synthesis and protein degradation. Total protein synthesis is, by definition, the product of the number of myonuclei and synthesis per nucleus.

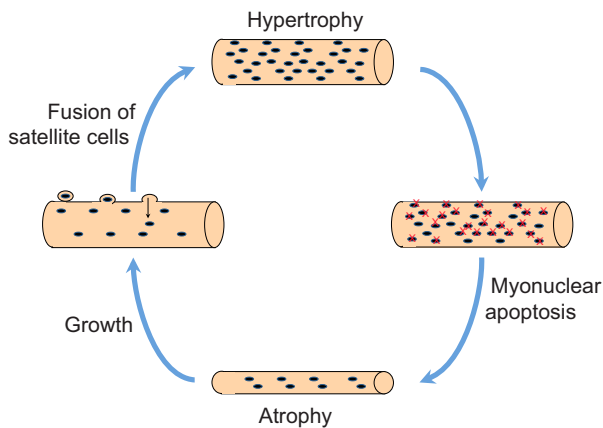


Fig. 2. Current textbook model for the cell biology of hypertrophy and atrophy. According to the model, when fibres grow as a response to a hypertrophic stimulus, myonuclei are recruited from satellite cells to support the larger cytoplasmic volume. During atrophy, the ‘excess’ nuclei are removed from the syncytium by selective nuclear apoptosis. In its strictest interpretation, the number of myonuclei are regulated such that the myonuclear domain volume is the same for the fibre in its atrophic and hypertrophic states. Note that the model is completely reversible; there is no apparent hysteresis or memory.

The controversies

Is recruitment of myonuclei obligatory for hypertrophy?

It seems generally accepted that myonuclei are added under many hypertrophic conditions (Allen et al., 1995, 1999; Bruusgaard et al., 2010; Cabric et al., 1987; Cabric and James, 1983; Cheek et al., 1971; Enesco and Puddy, 1964; Giddings and Gonyea, 1992; Kadi et al., 1999; Lipton and Schultz, 1979; McCall et al., 1998; Moss, 1968; Moss and Leblond, 1970; Roy et al., 1999; Schiaffino et al., 1976; Seiden, 1976; Winchester and Gonyea, 1992). However, the growth differs from the model shown in Fig. 2 in that the increase in number of myonuclei precedes the radial growth rather than lagging it, as has been demonstrated both with ^3H -thymidine labelling (Aloisi et al., 1973) and *in vivo* imaging (Bruusgaard et al., 2010). Thus, the myonuclear domain is temporarily decreased during the growth phase.

Although this time course might suggest that the increased number of myonuclei is causally related to the build-up of muscle mass, it has been debated whether the addition of myonuclei is obligatory for hypertrophic growth. According to literature from the early 1990s and onwards, hypertrophy is abolished or attenuated when satellite cells are ablated by X-ray irradiation (Adams et al., 2002; Barton-Davis et al., 1999; Phelan and Gonyea, 1997; Rosenblatt and Parry, 1992; Rosenblatt et al., 1994; but see also Lowe and Alway, 1999; Rosenblatt and Parry, 1993). However, this approach was criticized, as it was suggested that the irradiation might have affected other growth mechanisms in addition to ablating satellite cells.

After a comprehensive debate in the *Journal of Applied Physiology* in 2007 (Bodine, 2007; Hikida, 2007), it was concluded that limitations in current methodology made it difficult to reach final conclusions on whether an increase in the number of myonuclei is required for hypertrophy (O'Connor et al., 2007).

More recently, the debate was rejuvenated by several transgenic models displaying large fibres without a corresponding increase in the number of myonuclei, such as mice overexpressing the proteins Ski (Bruusgaard et al., 2005), Akt (Blaauw et al., 2009) or junB (Raffaello et al., 2010), as well as myostatin-null mice (Amthor et al., 2009). However, for Ski and myostatin the hypertrophy is not fully functional, as the specific force is reduced (Amthor et al., 2007; Charge et al., 2002; Mendias et al., 2011). Although it was not

measured, the same is probably true for junB, as it might also act by inhibiting myostatin (Raffaello et al., 2010).

Akt mice seemed to have normal specific force after 3 weeks of overexpression (Blaauw et al., 2009), but it is unclear whether this condition is sustainable over longer periods (Blaauw and Reggiani, 2014). Similarly, specific force appeared normal after 2 weeks of overload hypertrophy in mice where 90% of satellite cells were ablated using an inducible Pax7–diphtheria toxin transgene (McCarthy et al., 2011). However, the latter study is problematic because 30% of the fibres in the control material were regenerating, questioning the validity of the observations for pre-existing fibres. Also, I have calculated the average hypertrophy both in the control group and the group without satellite cells to be only 10% in the study, so the paper has little relevance to hypertrophy in excess of that. Even though the authors more recently published a long-term study (Fry et al., 2014), the interpretations for hypertrophy in pre-existing fibres are confounded by the high incidence of regenerative growth in the model used.

In conclusion, myonuclei are recruited before growth under many hypertrophic conditions, but it is still unclear whether one can also have a functional and sustainable hypertrophy without recruiting new myonuclei. As discussed in the next paragraph, nuclei are not lost during detraining or other atrophy conditions (the descending part of the model shown in Fig. 2). This is the core of the mechanism for muscle memory, and it allows for hypertrophic growth without recruitment of myonuclei during re-training, not because more nuclei are not needed in large fibres (as discussed above), but because the nuclei are already there.

Are myonuclei lost during atrophy?

Most of the focus on atrophy and atrophy prevention has been on satellite cells and their activation, i.e. multiplication and differentiation into myoblasts. Based on the model shown in Fig. 2, atrophy was interpreted as a degenerative disease where myonuclei need to be replenished, for example, by stem cell treatment. Some authors have even blurred the conceptual distinction between myonuclei proper and satellite cells, and are talking about muscle nuclei. This is unfortunate, because the functionally important nuclei for synthesis of muscle proteins are the myonuclei, not the satellite cells. Thus, the ability to distinguish the myonuclei both conceptually and physically becomes crucial. Precise identification of myonuclei is not trivial because at least half of the nuclei of muscle tissue are in other cell types. The descending limb of the model in Fig. 2 has become highly controversial, and previous misconception is likely to be largely due to difficulties in identifying myonuclei.

Using *in vivo* time-lapse imaging where myonuclei in specific segments of the same muscle fibre could be followed over time (Bruusgaard and Gundersen, 2008), we observed that the number of myonuclei remained constant when fibres were followed for up to 28 days after denervation, resulting in a volume loss of more than 50%. One example is shown in Fig. 3. Similar observations were made for both fast and slow muscle, and in several different atrophy models such as nerve impulse block with tetrodotoxin, antagonist ablation (Bruusgaard and Gundersen, 2008), hind limb suspension (Bruusgaard and Gundersen, 2008) and detraining (Bruusgaard et al., 2010).

Although *in vivo* imaging represents direct observation of myonuclei, several papers (e.g. Alway et al., 2003a; Ferreira et al., 2006; Pierce et al., 2008; Siu and Alway, 2005; Siu et al., 2005; Tang et al., 2000) have found markers for apoptosis in atrophying muscle homogenates and have interpreted this as apoptosis of myonuclei, but clearly this could reflect apoptosis of non-myonuclei.

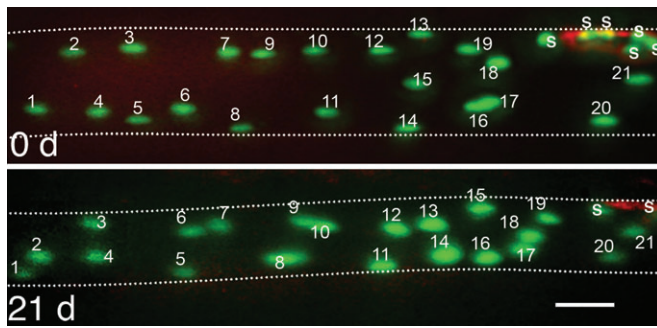


Fig. 3. Myonuclei are not lost during atrophy. Time-lapse *in vivo* imaging of the same fibre segment before (day 0) and after 21 days of denervation. Note that the number of nuclei in the segment left of the neuromuscular endplate labelled in red is the same in spite of the pronounced atrophy. Scale bar, 50 μm . Adapted from Gundersen and Bruusgaard (2008).

There is also a large literature based on histological analysis using conventional staining techniques stating that nuclei are lost by apoptosis during atrophy (e.g. Adhietty et al., 2007; Allen et al., 1997; Alway et al., 2003b; Meneses et al., 2014; Tews et al., 1997; Yoshimura and Harii, 1999). However, with such a technique it is difficult to distinguish between nuclei of different cell types, in particular satellite cells, which are located under the basal lamina of the muscle fibres.

Newer studies have used antibodies against the myofibre cytoskeleton protein dystrophin to outline the fibre surface as a way to separate myonuclei from the nuclei of satellite and stroma cells. Even this literature has yielded conflicting results, and the precise rules for deeming a nucleus to be inside or outside the dystrophin ‘ring’ on cross-sections seems to be crucial for the result. Unfortunately, these rules are generally poorly described in the literature.

In our studies we have defined myonuclei as nuclei with their geometrical centre inside the inner rim of the dystrophin ring (Bruusgaard et al., 2012, 2010; Bruusgaard and Gundersen, 2008; Gundersen and Bruusgaard, 2008). Using this definition, the averages are all below 1.7 myonuclei per fibre on 8–10 μm cross-sections. When the same operator in our laboratory evaluated the same sections, but also included nuclei with their geometrical centre on the dystrophin ring, the number of ‘myonuclei’ increased by 85% (Bruusgaard et al., 2012), and it is questionable whether these were all myonuclei. Using the strict definition, however, we have essentially never observed apoptotic myonuclei under atrophy conditions (Bruusgaard et al., 2012, 2010; Bruusgaard and Gundersen, 2008; Gundersen and Bruusgaard, 2008).

Others using dystrophin staining have also described myonuclear apoptosis and/or loss of myonuclei during atrophy (Dupont-Versteegden et al., 2006; Guo et al., 2012; Leeuwenburgh et al., 2005; Oishi et al., 2008; Zhu et al., 2013). However, they reported a >30% higher number of defined myonuclei (>2.2 myonuclei per fibre) on 6–10 μm cross-sections (Dupont-Versteegden et al., 2006; Leeuwenburgh et al., 2005; Oishi et al., 2008), suggesting that the population of nuclei included was considerably less selective than in our studies.

In addition to the problem of identifying myonuclei, the terminal deoxynucleotidyl transferase dUTP nick end (TUNEL) staining used to label apoptotic nuclei is prone to false positives (Garrity et al., 2003; Pulkkanen et al., 2000). With a labelling incidence of 1.5 nuclei per section in normal control material from rat limb (Gundersen and Bruusgaard, 2008), virtually no apoptotic myonuclei appeared upon denervation, while many apoptotic nuclei appeared in mononuclear cells (Gundersen and Bruusgaard, 2008). In contrast, a report

displaying seven-times higher incidences of TUNEL-positive nuclei in comparable control material reported a loss of myonuclei by apoptosis (Dupont-Versteegden et al., 2006).

Several studies based on fibres isolated *ex vivo* after denervation have also concluded that myonuclei are not lost during atrophy (Aravamudan et al., 2006; Bruusgaard et al., 2012, 2010; Duddy et al., 2011; Wada et al., 2002). Duddy et al. (2011) used cultured, live fibres and identified myonuclei very precisely using a muscle-specific reporter gene. Wada et al. (2002) and Bruusgaard et al. (2010, 2012) isolated fibres by alkaline maceration. When Wada et al. (2002) compared this method with mechanical isolation, they found that mechanical isolation was unreliable because of both adhering non-myonuclei and loss of myonuclei from the fibres. This might explain why a loss of myonuclei has been reported after mechanical isolation (Viguie et al., 1997). Similarly, Kawano et al. (2008) reported a loss of myonuclei after collagenase digestion, but their myonuclear counts were three to four times higher than those observed by *in vivo* imaging, suggesting that adherent cells were also a problem with this technique.

In conclusion, by direct observation with *in vivo* imaging one observes no loss of myonuclei during atrophy, and this is confirmed by observations of isolated single fibres *ex vivo* when contamination of non-myonuclei adhering to the fibres is minimized. These observations are also confirmed by the histological studies with the most conservative inclusion criteria for myonuclei. Similarly, the studies with the least risk of false positive TUNEL-labelling have failed to demonstrate apoptotic myonuclei. Thus, there is currently no compelling reason to believe that myonuclear apoptosis occurs under atrophy conditions. One might question whether a selective apoptosis of some nuclei within an intact syncytium ever takes place or is even possible. This does not exclude the possibility that whole fibres or fibre segments could be eliminated by apoptosis, but this is not relevant for the atrophy of pre-existing fibres.

What determines the number of myonuclei?

The conclusion that myonuclei are recruited during hypertrophy and not lost during subsequent atrophy seems to suggest that by going through repeated hypertrophy/atrophy cycles one could ‘pump up’ the number of nuclei indefinitely. This seemed implausible, and we (Bruusgaard et al., 2012) therefore reinvestigated the fate of myonuclei in the hind limb suspension model where atrophy is induced by lifting up the hind part of rats by the tail and thereby unloading the hind legs. Using *in vivo* imaging, we observed that myonuclei were not lost during the resulting atrophy. When the muscles were reloaded by letting the animal back down again, the fibres displayed a 60% radial growth, and this growth was not accompanied by any increase in the number of myonuclei. Such growth also does not seem to require the presence of satellite cells (Jackson et al., 2012). Thus, we suggest that hypertrophy can occur without new myonuclei, provided the myonuclear number is already high.

Based on data from human studies, the so-called ‘ceiling hypothesis’ has been put forward. It states that until a certain limit of hypertrophy is reached, hypertrophy can occur without recruiting new myonuclei (Kadi et al., 2005). The limit has been defined as hypertrophy above a certain per cent (e.g. 17–36%) (Kadi et al., 2005) or as an absolute myonuclear cytoplasmic domain volume that, when exceeded, triggers recruitment of myonuclei (Petrella et al., 2006, 2008).

We suggest that the number of myonuclei not only reflects the current size of the fibre as implied by the ceiling hypothesis, but also the history of the fibre. Current data might fit a ‘peak pegging’

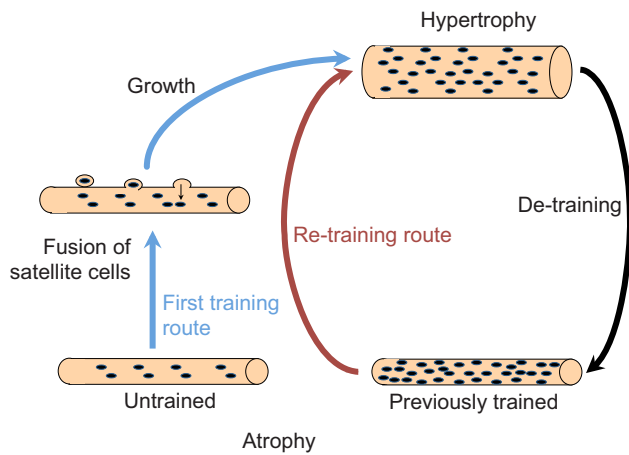


Fig. 4. A new model for the cell biology of hypertrophy and atrophy. For naive fibres and preceding hypertrophic growth, myonuclei are recruited from satellite cells, temporarily reducing the myonuclear domain volume, leading to a large fibre with many myonuclei. Upon subsequent atrophy the myonuclei are maintained, leading to a small fibre with a high myonuclear density and small myonuclear domains. Such fibres can hypertrophy without recruiting new nuclei, and this re-training route seems to be faster than the first training route. The permanently higher number of myonuclei represents the muscle memory. Adapted from Bruusgaard et al. (2010).

hypothesis, where the number of myonuclei found in a fibre represents the largest size the fibre has had in its history, and new myonuclei are only added if the fibre grows beyond that size. This would be analogous to a conventional minimum/maximum thermometer, where the mercury column pushes a peg upwards and leaves it at the highest temperature measured.

A new model for muscle size regulation

As concluded above, myonuclei seem to be recruited during overload hypertrophy, and not lost during subsequent atrophy (see particularly Egner et al., 2013). Thus, a previously hypertrophied fibre would differ from a naive fibre in having more nuclei as a ‘memory of its past’. Technically, the fibre displays hysteresis: the cyto-architecture depends on previous exposure. This observation led us (Bruusgaard et al., 2010) to propose the model for the cell biology of muscle size regulation illustrated in Fig. 4. In this model,

previously untrained fibres are small with few nuclei; when subjected to a hypertrophic stimulus they acquire new nuclei through a ‘first training route’. These new nuclei precede the growth in time and might be causally related to the subsequent fibre enlargement. The end product is a large fibre with many nuclei. Upon subsequent detraining, the fibres maintain the elevated number of nuclei, but lose protein resulting in a small fibre with a high number of nuclei. If the fibre is re-trained from this state we suggest that a different re-training route can be followed, skipping the step of recruiting myonuclei. The next questions we asked were whether this re-training route is beneficial, and whether it is easier or faster than the first training route.

Muscle memory

The re-training route is faster

To investigate whether there is a beneficial muscle memory, Egner et al. (2013) treated female mice with testosterone for 2 weeks. As illustrated in Fig. 5, this led to an increase in both fibre size and the number of nuclei. Three weeks after drug withdrawal, the fibre size was back to normal, but the number of nuclei remained constant and high. Three months later (>10% of the mouse lifespan), the number of nuclei was still essentially maintained. When at this time the muscles were subjected to overload, the high-nuclei muscles grew by 36%, while the control group grew insignificantly by only 6%. After that, the groups grew in parallel, but the high-nuclei group remained larger for the duration of the 2-week overload experiment. Egner et al. (2013) concluded that the re-training route seems to allow faster growth than the first training route, and suggest that the memory storage mechanism is the number of nuclei.

How lasting is the muscle memory?

We have demonstrated that in mice, the memory effect lasts for >10% of the animal’s lifespan. In humans, the turnover of cells has been studied by utilizing the peak in ¹⁴C availability after the post-war atmospheric testing of nuclear bombs (Spalding et al., 2005). Intercostal muscle tissue was harvested from two individuals (age 37–38 years) and the ¹⁴C content in genomic DNA indicated an average age of the nuclei of 15.1 years. For several reasons this is a low-end estimate of the possible lifespan of human myonuclei. First, approximately half of the nuclei in the tissue are in other cell types, mostly with a higher turnover. By comparison, in gut approximately

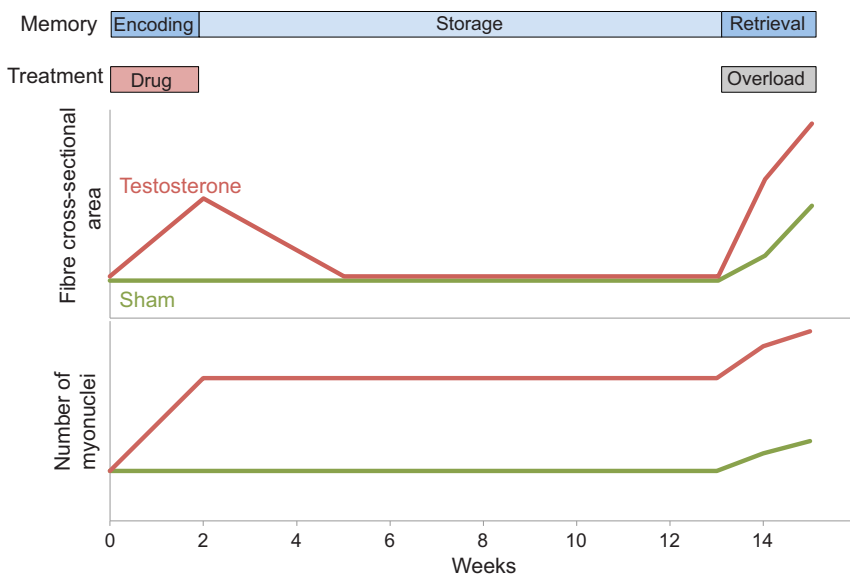


Fig. 5. Demonstration of beneficial muscle memory in mice. Schematic representation of an experiment (Egner et al., 2013) demonstrating the encoding, storage and retrieval of muscle memory. The encoding occurred during a brief exposure of the animals to testosterone, which led to an increase in fibre size and the number of myonuclei. When the drug was removed, fibre size reverted to control levels of sham-treated animals, but the high number of myonuclei was retained (storage). When both the testosterone and the sham groups were subjected to overload 3 months later, the former group grew much faster than the latter group (retrieval).

40% of the cells are epithelial with a turnover rate measured in days; when these were excluded the average lifetime of nuclei was increased by 50% in that tissue. Second, the measurement was from young individuals that had been growing for a significant part of their life and thus creating new myonuclei. Third, new myonuclei might have been created during relatively recent strength exercise.

It is possible that myonuclei are more or less permanent once created; for example, human brain cortical neurons appeared to be as old as the individual (Spalding et al., 2005). For cardiomyocytes, 1% turn over annually at an age of 25 years, gradually decreasing to 0.45% at an age of 75 years. It was calculated that fewer than 50% of cardiomyocytes are exchanged during a normal lifespan (Bergmann et al., 2009). In contrast to the brain and heart, however, muscle tissue is subjected to repair after damage with new nuclei from satellite cells. How much damage and repair normally accumulates over decades is, however, unknown. Thus, with current knowledge it is hard to be more precise than estimating the lifespan of myonuclei, and hence the muscle memory span, to somewhere between 15 years and a human lifetime.

Evolutionary considerations

One might speculate as to the evolutionary origin of muscle memory. Based on the original model (Fig. 2), it was speculated that nuclei were lost during atrophy because they might be expensive to maintain. ‘Cost’ might be energy or space; muscle fibres are optimized with respect to packing contractile proteins. However, the cost of keeping a high number of nuclei in a small fibre has never been quantified, nor compared with the cost of eliminating and recreating myonuclei with ever-changing demands for muscle strength.

In the new model (Fig. 4), the muscle memory might represent an adaptive mechanism to allow individuals from whom strength has been demanded in the past to more quickly rebuild muscle mass in the future, as an individual adaptation or specialization. In this way, maintaining a constant high muscle mass is avoided, for example, during less labour-intensive seasons. As for the classical learning and memory of the brain, ‘experience’ becomes useful if the same task arises again.

Implications for sport and public health

Healthy ageing

Decline in muscle strength is a major health problem in the ageing western population (Dutta and Hadley, 1995; Hughes and Schiaffino, 1999), and more than 50% of individuals currently fill the clinical criteria for frailty at ages >80 years (Matthews et al., 2011). Hypertrophy induced by overload is greatly attenuated in older animals (Alway et al., 2002; Carson et al., 1995), and the ability to generate new myonuclei is impaired (Schultz and Lipton, 1982), possibly because of reduced notch signalling in the elderly (Conboy et al., 2003; Conboy and Rando, 2005). The role of a similar nuclei-related muscle memory in humans should be investigated. Such knowledge might lead to public health advice for strength training to recruit new myonuclei in younger individuals, as these myonuclei might aid in maintaining muscle mass more easily in senescence.

Doping

Muscle memory was induced in mice by testosterone, and it was demonstrated that brief exposure to this hormone aided hypertrophy induced by overload long after the drug was removed (Egner et al., 2013). If this is applicable to humans, it must have consequences for doping rules. According to Anti-Doping Norway, animal

experiments on the permanency of recruited myonuclei (Bruusgaard and Gundersen, 2008) have already contributed to increasing the exclusion time for doping offences from 2 to 4 years. Given the longevity of human myonuclei, this would not be sufficient to ensure that previous steroid use does not still give a competitive advantage when the exclusion time is over. The position of the World Anti-Doping Agency has been that no regulatory action should be taken before the muscle memory phenomenon is confirmed in humans. This raises interesting questions about the burden of proof for convicted cheaters. Nonetheless, if confirmed in humans, the possibility of a long-term muscle memory induced by a brief steroid exposure raises serious questions about the possibility of policing a drug-free sport.

Future perspectives

Although there is evidence for the new model shown in Fig. 4 and for muscle memory induced by testosterone in rodents (Fig. 5), it should also be demonstrated that muscle memory could be encoded by strength exercise without steroids. As for humans, although anecdotal evidence suggests the existence of training-induced muscle memory, this needs to be confirmed in controlled exercise studies.

Although the demonstrated long-lasting increase in the number of myonuclei is a logical epigenetic substrate for the memory phenomenon, it does by no means exclude other mechanisms. DNA and histone modifications are now widely accepted for gametogenesis and early cell differentiations. There is also emerging evidence that such mechanisms occur in somatic, fully differentiated cells such as muscle cells, and that they might play a role in the malleability of muscle phenotype in response to exercise (Rasmussen et al., 2014).

Competing interests

The author declares no competing or financial interests.

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