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Perspective Article

Is the myonuclear domain size fixed?

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Abstract

It has been suggested that the number of myonuclei in a muscle fibre changes in proportion to the change in fibre size, resulting in a constant myonuclear domain size, defined as the cytoplasmic volume per myonucleus. The myonuclear domain size varies, however, between fibre types and is inversely related with the oxidative capacity of a fibre. Overall, the observations of an increase in myonuclear domain size during both maturational growth and overload-induced hypertrophy, and the decrease in myonuclear domain size during disuse- and ageing-associated muscle atrophy suggest that the concept of a constant myonuclear domain size needs to be treated cautiously. It also suggests that only when the myonuclear domain size exceeds a certain threshold during growth or overload-induced hypertrophy acquisition of new myonuclei is required for further fibre hypertrophy.

Keywords: Skeletal Muscle, Myonuclear Domain, Satellite Cell, Hypertrophy, Atrophy, Ageing, Growth

Introduction

Skeletal muscle has a remarkable ability to respond to altered functional demands. During disuse, for instance, the muscle atrophies, while in response to overload the muscle hypertrophies. Whatever the condition that causes muscle fibre atrophy or hypertrophy, the speed and magnitude of this change in size are the result of an altered balance between protein synthesis and degradation. Although protein synthesis and degradation are regulated by different sets of complex independent pathways, there are indications of some interaction between the two, where specific proteins that stimulate protein synthesis also inhibit the expression of proteins that increase the activity of protein degradation pathways[1,2]. Protein degradation is executed by the interaction of several different proteolytic pathways: 1) the cytosolic calcium-dependent calpain system, 2) the lysosomal proteases, 3) the ATP-dependent ubiquitin proteasome system[2-4] and 4) caspases[5]. The rate of protein synthesis can be regulated by: 1) the rate of protein translation, 2) the amount of available mRNA and 3) the transcriptional capacity.

The translational capacity is determined by the number and translational efficiency of ribosomes[6]. Given that the fraction of ribosomal RNA accounts for approximately 80-85% of the total cellular RNA pool[7], changes in the total RNA concentration are generally regarded to reflect a change in the translational capacity[6]. The transcriptional capacity is determined, at least partly, by the amount of available nuclear DNA. While most cell types (e.g. neuronal cells, macrophages, fibroblasts, lymphocytes, osteocytes) in mammals are mono-nucleated, muscle fibres contain multiple nuclei (Figure 1). Single soleus muscle fibres from 5-month-old rats, for instance, contain approximately 300 myonuclei per mm muscle fibre length[8]. Those nuclei originate from the fusion of multiple myoblasts into one myotube[9] during embryonic development. Muscle nuclei, further indicated as myonuclei, have a flattened and elongated shape and their length varies between 11-25 μm e.g.[10,11-15] and does not vary significantly with age and denervation-induced atrophy (Figure 2)[14].

For more than a century scientists have had the idea that each nucleus has a 'Wirkungskreis' or 'volume of influence'[16]. The general believe was that nuclear size was related to cytoplasmic volume. In other words, it was thought that the size of mononuclear cells was largely determined by the size of the

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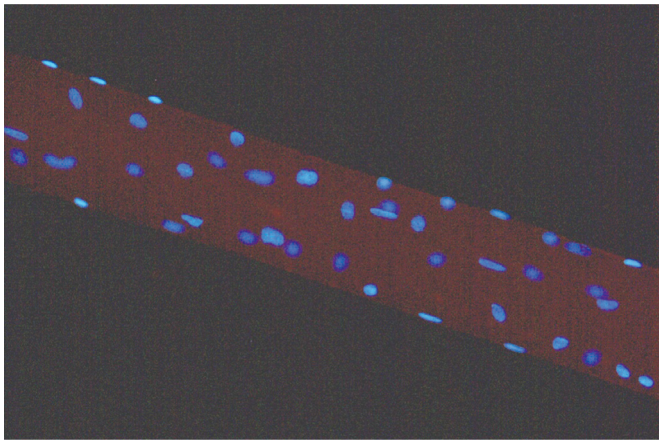

Figure 1. Fragment of a single human vastus lateralis muscle fibre showing in blue the myonuclei.

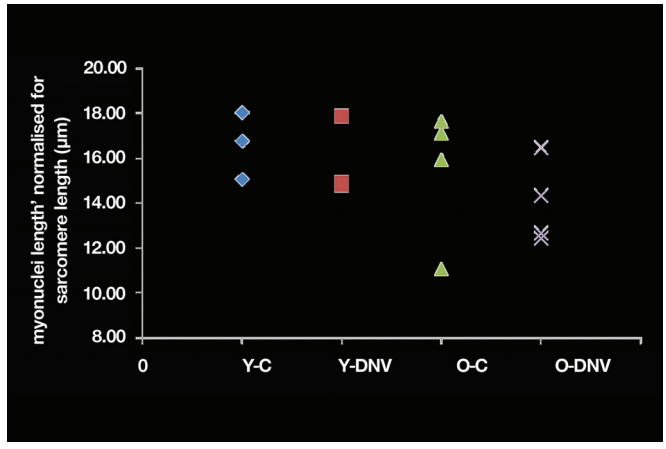

Figure 2. Myonuclear length determined in single isolated fibres from young-adult control (Y-C), young-adult 4-week denervated (Y-DNV), old control (O-C) and old 4-week denervated (O-DNV) rat gastrocnemius muscles (3 fibres from 3-4 muscles in each group). Myonuclear length (in µm) was estimated as the longest length of a best-fit ellipse. The angle of each myonucleus was corrected for the angle at which the muscle fibre fragment was captured. In three different regions of the fibre the mean sarcomere length was determined to correct nuclear length for sarcomere length (hence the y-axis presents myonuclear length normalized for sarcomere length which was in all cases close to 2.4 µm) and orientation of the nucleus within the fibre. Each data point represents the mean of 21±3.4 (mean±SEM) myonuclei. Using a two-way ANOVA, it appeared that age and denervation did not have a significant effect on myonuclear length (unpublished observations).

myonucleus[17]. Only in the 1960s scientists became interested in the relationship between cell size and number of nuclei in skeletal muscle[18-20]. It was, however, Cheek and colleagues who in 1971 hypothesised that 'each nucleus held jurisdiction over a finite volume of cytoplasm' and developed the concept of a 'DNA unit'[20]. A functional DNA unit was defined as the content of non-collagen-protein per DNA content. It should be noted, however, that by measuring the DNA content in a muscle homogenate also nuclei originating from other cell types (satellite cells, fibroblasts, endothelial cells etc.) are included. A more accurate assessment of the relationship between number of nuclei and the muscle fibre size would be based on a count of the myonuclei in a muscle fibre.

Landing and colleagues, using single muscle fibres, showed for the first time that the distribution of the nuclei in muscle fibres follows regular patterns; each nucleus was surrounded by six other nuclei in an "elongated hexagon" further supporting the idea that each myonucleus has a "surface territory" as indicated by the "cytoplasmic-volume-to-nucleus ratio"[21]. Edgerton and Roy[22] pointed out that a myonucleus can theoretically act in two scenarios as illustrated in Figure 3. According to the model represented in Figure 3A and described by Cheek[20] and Hall and Ralston[23] a myonucleus provides all transcripts for a given volume of cytoplasm in its direct surrounding. In the model of Figure 3B each myonucleus expresses only specific proteins, but does so for the whole muscle fibre. Many observations support the first concept. Some proteins are not homogeneously expressed over the full length of the muscle fibre[24]. In addition, considering the limited half-life of mRNA and relatively large dimensions of a muscle fibre (they can be several cm long and up to 0.2 mm in diameter), it seems unlikely that muscle fibres could maintain their size if transcripts must diffuse over long distances within the fibre[22]. Indeed mRNA does not range far from its nucleus of origin[25,26]. Besides, the concept of a myonuclear domain can only be useful when one assumes that each myonucleus has a certain spatial limitation to control its surrounding cytoplasm[22].

Further support for the concept that each myonucleus has a limited cytoplasmic volume which it can supply is the observation that under normal physiological conditions the number of myonuclei per muscle fibre is proportionally positively related to muscle fibre size in both animal[15,27-29] and human muscle[30,31]. If this concept of a constant myonuclear domain size is true, then any change in the size of a fibre will be accompanied by a proportional change in the number of myonuclei in that fibre: the possible scenarios are illustrated in Figure 4B-E.

In this review we will explore the validity of this concept by looking at reported changes in myonuclear domain size during maturational growth, hypertrophy, atrophy and ageing.

The myonuclear domain in fibres of different type

The myonuclear domain size appears to differ between fibre types, where it increases from type I to IIA to type IIB/X fibres[14,29]. In rat gastrocnemius muscle the larger myonuclear domain size in the type IIA than type I fibres was due to a lower number of myonuclei, and not a larger size of the IIA fibres. In contrast, in type IIB/X fibres the number of myonuclei in the fibre was similar to that of type I fibres but type

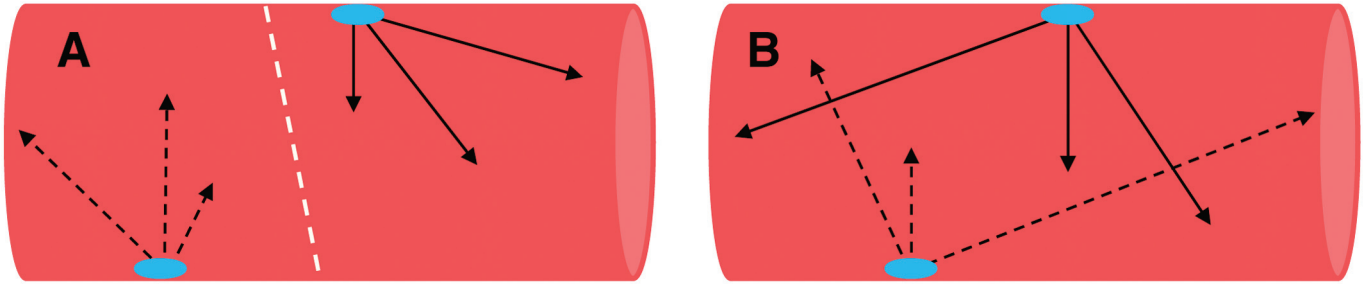

Figure 3. Two concepts of myonuclear domain. The arrows indicate the domain of cytoplasm that the myonuclei supply with mRNA transcripts. In the left diagram (**A**) the myonucleus provides the cytoplasm in its direct surrounding of transcripts, whereas in the right diagram (**B**) it is assumed that each nucleus produces only specific transcripts which diffuse through the whole muscle fibre.

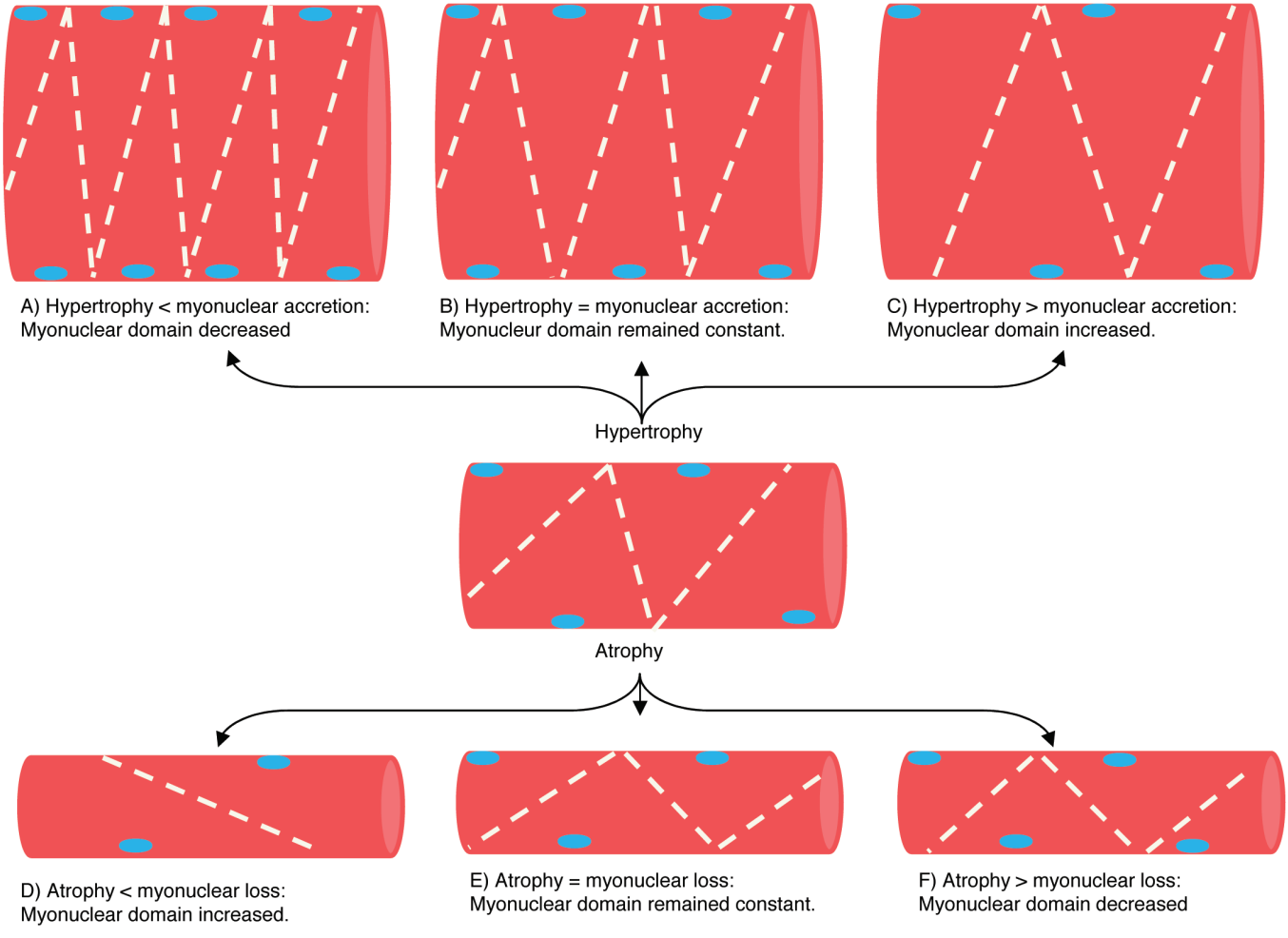

Figure 4. Possible scenarios of changes in myonuclear domain size with different types of muscular adaptation.

IIB/X fibres had a larger fibre cross-sectional area (FCSA)[14]. These differences in myonuclear domain size between fibre types could well be related to the higher oxidative capacity of the type I and IIA than the type IIB/X fibres since the myonuclear domain size is inversely related to the oxidative capacity of the fibre[15,32]. Further support for the notion that the myonuclear domain size is related to the oxidative capacity of muscle fibres rather than the myosin heavy chain type of the fibre is

provided by the observation that the type II fibres in the superficial glycolytic region of the rat plantaris muscle with a relatively low oxidative capacity had a larger myonuclear domain than type II fibres in the deep oxidative region of the plantaris muscle with a high oxidative capacity[33]. A possible explanation for the differences in myonuclear domain size between fibres of different types may be that oxidative fibres have a faster protein turnover and hence a higher demand for mRNA transcription, also of mitochondrial proteins. It has indeed been observed that protein turnover rate is higher in slow than in fast muscles[2,34]. Overall, it signifies that the myonuclear domain size is not fixed but does vary between fibre types and with the oxidative capacity of the fibre.

Myonuclear domain and increases in muscle size

Skeletal muscle growth is largely, if not solely, attributable to an increase in fibre size. According to the concept of the myonuclear domain this should be associated with a proportional increase in the number of myonuclei to maintain the myonuclear domain size. Below we will discuss what happens during maturational growth of the muscle and exercise- or overload-induced muscle growth or hypertrophy.

An increase in the number of myonuclei is realised by the fusion of satellite cells with the existing muscle fibre(s). Satellite cells were discovered by Mauro[35] who named them after their location between the plasma membrane of the muscle fibre and the basement membrane (also referred to as 'basal lamina') that surrounds the muscle fibre. Once activated the expression of the myogenic regulatory factor MyoD is increased and the cell starts to divide. During this proliferation phase the expression of the myogenic regulatory factors will shift from MyoD to myogenin, which is thought to be crucial for satellite cell differentiation. At fusion with the muscle fibre, the satellite-cell nucleus is donated to the existing muscle fibre and will function as a myonucleus. It should be noted, however, that not all activated satellite cells differentiate into a myonucleus. After proliferation some satellite cells are withdrawn from the differentiation process[36]. It is hypothesised that those satellite cells fall back to a quiescent state and maintain a pool of satellite cells that can be activated during later repair, regeneration or hypertrophy[36].

Maturational muscle growth

During maturational growth, the lengthening of the bones is tightly associated with increases in skeletal muscle volume. An expansion of muscle volume can be achieved by increases in the number, length and/or cross-sectional area (FCSA) of the muscle fibres. Since it is generally believed that the number of muscle fibres is set at around the first months after birth[37,38], the increase in muscle volume during growth must be the result of an up to 23-fold increase in fibre size[39-41] and an increase in muscle fibre length[42,43].

In 1964 Enesco and Puddy reported that the number of myonuclei increased during the first three months of growth in rats, but did not formulate any hypotheses on the relevance of a possible coupling between the cell size and the number of nuclei[44]. In the pectoralis and gastrocnemius muscles from growing chickens a logarithmic relationship was observed between the number of nuclei, estimated by the DNA content, and the muscle fibre diameter[18,19]. They relied thereby on earlier findings that the DNA-per-nucleus ratio in various mammalian cells was a constant value of about 6.2 pg per nucleus[20]. In the early reports of nuclear increases during muscle growth[18,44] myonuclei were not discriminated from other nuclei. Although Enesco and Puddy demonstrated that the fraction of muscle nuclei, nuclei that were considered inside a muscle fibre, remained fairly stable (60-70%) in each of four different rat muscles during their development from 16 to 89 days, those reports should be interpreted with some caution.

Nevertheless, these early studies were subsequently confirmed by additional observations of a substantial increase in the number of myonuclei per muscle fibre during the early weeks and months of growth in rodents[8,42,45,46]. However, the increase in myonuclear number was shown to be less than the increase in FCSA and consequently the myonuclear domain size expands during growth. The expansion of the myonuclear domain size during maturational growth in rat diaphragm muscle fibres was more pronounced in type IIX than in IIA or type I muscle fibres[46]. This suggests that, at least in rat, the growth of type IIX fibres requires a smaller transcriptional capacity than is the case for the type I and IIA muscle fibres and corresponds with a lower protein turnover rate in fast than slow fibres[2,34], requiring a lower transcriptional activity.

Little is known about changes in myonuclear number during growth in human muscle, but the few data available suggest that the pattern observed in rodents and chicken also applies to human muscle. Muscles of children in the age of 1-2.5 years old contained approximately half the number of myonuclei per muscle fibre of those from adult muscles (12-30 years old)[41]. At first glance the proportional increase in myonuclei and myofibre diameter suggests that the nuclear domain size remains constant. However, FCSA increases quadratically with diameter and hence it is clear that also in this case there is an increase in the myonuclear domain size during growth. It is unclear, however, what happens in the first few months after birth. As the number of satellite cells declined rapidly during the first weeks of growth of rat skeletal muscles[12,42,47,48], it is no surprise that myonuclear accretion slows down during maturation[42].

Overall the changes in FCSA and nuclear number during growth resemble the situation depicted in Figure 4C where the nuclear domain size is not constant but rather increases during maturational muscle growth. It thus seems that the concept of a constant nuclear domain size needs to be abandoned at least during maturational muscle growth. Maturational growth is a special situation where the whole body, including the muscle, is geared to subsequent growth. This requires an exceptional transcriptional activity which is undoubtedly much higher than one would see in the adult myofibre that finds itself in a more or less steady state. It is interesting to note, however, that the increase in myonuclear domain size as a consequence of a larger increase in FCSA than nuclear number much resembles the larger in-

Reference	Species	Age	Model	Duration (days, months)	N	Muscle	change MN (%)	change FCSA (%)	change MD (%)
29	R-SD♀	180 g	Overload	10 weeks	3	PL	61-109	36-90	=
33	R-W♂	5 mo	Overload	28 days	5	PL	↑	33	=
		25 mo			3		↑	33	=
51	R-SD♀	212 g	Overload	90 days	6	PL	44	46	=
			Overload+IR		6		=	=	=
56	R-W♂	152 g	Overload	28 days	6	EDL	ND	21 (ww)	=
			Overload+IR		6			-24 (ww)	19
57	R-W♂	4-5 mo	Overload	28 days	8	EDL	ND	18 (ww)	=
		(450-500 g)	Overload IR		8			= (ww)	=
54	H♀	28	RT	16 weeks	13	VL	=	27	17
		63			13		=	17	16
54	H♂	26	RT	16 weeks	13	VL	19	27	10
		65			13		=	17	10
59	H♂	24	RT	90 days	15	VL	=	17	17*

*H: Human; R: Rat; ♀: Female; ♂: Male; W: rat strain Wistar; SD: rat strain Sprague-Dawley; Age in years for human and in mo (months) for rats; (N): number of participants / animals; MN: myonuclear number; FCSA: fibre cross-sectional area; MD: myonuclear domain; ww: % change in wet weight; Muscles: VL: vastus lateralis; PL: Plantaris; EDL: Extensor digitorum longus; IR: irradiation; All changes have been expressed as change in hypertrophied compared to control muscles; =: no significant change with hypertrophy; *:change estimated from authors graph.*

Table 1. Studies investigating the effect of hypertrophy on the myonuclear domain size.

crease in FCSA than capillary number during growth, resulting in an increased capillary supply or 'capillary domain' area[39]. Also in that case, like the number of myonuclei[41], the capillary number per fibre radius remained constant[39] suggesting that the average distance between myonuclei remains relatively constant during growth. The significance of this distribution of myonuclei is something for further exploration.

Hypertrophy

It is a point of discussion whether or not the acquisition of new myonuclei is required for skeletal muscle hypertrophy[49,50]. Table 1 gives an overview of the changes in myonuclear domain size during hypertrophy. At a first glance, the increase in the myonuclear number during hypertrophy in animals[29,33,51-53] and young-adult men[54] appears to support the notion of a constant myonuclear domain size. The importance of myonuclear addition for muscle fibre hypertrophy is further supported by the attenuated[55,56] or even prevented hypertrophy after 'gamma-irradiation'[51,57,58], which destroys the potential of mitotic division in various cell types, including the satellite cells. However, it has been shown that hypertrophy to some extent is possible without any change in the number of myonuclei[33,54,59,60]. Using a cluster-analysis of a 4-months resistance training program in humans, it was observed that participants who responded with only moderate (9.7±2.4%) hypertrophy were those where the number of myonuclei per muscle fibre did not increase and the myonuclear domain size remained below 2,000 μm^2 [54]. This value was proposed as a 'ceiling' of the myonuclear domain size beyond which additional hypertrophy can only be realised by addition of new myonuclei[54]. The proposed values of a hypertrophy threshold or a ceiling of the myonuclear domain size are somewhat arbitrary. In this perspective it is interesting to note that recently for a variety of species it has been shown that myonuclear domain size scales in proportion to body mass[61]. Thus, a myonuclear domain size ceiling or hypertrophy threshold may vary between species.

There is, however, a lack of knowledge regarding the time course of changes in the number of myonuclei during hypertrophy[62]. In one such study significant hypertrophy of the plantaris muscle was attained after 2 weeks of overload without a concomitant increase in myonuclear number[33]. Consequently, the myonuclear domain size increased and only after 4 weeks of overload did the number of myonuclei increase[33]. This observation corresponds with the notion of a ceiling of hypertrophy where further hypertrophy after 2 weeks of overload requires the addition of new myonuclei. A recent study casts some doubt on this conclusion as they found a stunning doubling in muscle size after only two weeks of overload in satellite cell depleted mice[63]. Although one could argue that the muscle fibres would be of lower functional capacity the authors reported normal single muscle fibre function. Apparently the myonuclei do have a reserve capacity. The acquisition of new myonuclei does take time and requires proliferation of satellite cells, that can be detected as early as 1 week after induction of overload[58], and subsequent differentiation into a myonucleus. This may only result in a significant increase in the number of myonuclei after more than 2 weeks of overload[33], emphasising the need for a reserve capacity of the myonuclei to cope with sudden changes in demand for mRNA transcripts. It is thus possible that doubling in muscle size within two weeks in satellite

Reference	Species	Age (months)	Model	Duration (days, months)	N	Muscle	change MN (%)	change FCSA (%)	change MD (%)
Bed rest									
101	H, ♂	31 yrs	B	2 mo	6	VL	=	=	=
				4 mo	6		=	-35	-35*
Denervation									
74	R-SD, ♂	adult	DNV	14 d	5	Diaph, I	=	+15	+26
						Diaph, IIA	=	=	=
						Diaph, IIX	=	-46	-51
						Diaph, IIB		-67	-69
75	M[1]	4	DNV	10 d	-	PL	=	-43*	-43*
				4 mo			=	-58*	-54*
11	R-W, ♂	1.5	DNV	2.5 mo		EDL	-17[2]	-95	-
102	R-W, ♀	170-200 g	DNV	7 d	-7 fibres	SM	=	-	-37
				14 d			=	-	-60
103	M-NMRI, ♀		- DNV	7 d	-	EDL	=	=	-
				14 d			=	-29*	
				21 d			=	-51	
				14 d		SOL	=	-42*	
				21 d			=	-55	
104	R-WI/Hick, ♂	4	DNV	20 mo	3	EDL	-36	-86*	-88
				4 mo	3		-53	-97*	-96
				7 mo	3		-68	-98*	-92
11	R-W, ♂	1.5	DNV	2.5mo		SOL	-60	-99	-
14	R-W, ♂	5	DNV	7 d	5	Gast	=	-47	- ca 45
Spinal cord injury									
71	R-SD, ♀	adult	SI	4 d	5	SOL, I	=	-41	-36
				2 mo	5		-25	-66	-55
68	R-SD, ♀	adult	SI	10 d	4-5	SOL	-25	-	-
53	C, ♀	adult	SI	6 mo	3	SOL, I	=	-66	-64
						SOL, II	-32	-74	-60
Hindlimb suspension									
97	R-F344.BN, ♂	6	HLS	7 d	6	SOL	=	-24	=
				14 d	6		-36*	-62	-41*
52	R-W, ♂	3	HLS	14 d	8	SOL	-31	-50	-28
73	R-W, ♀	3	HLS	14 d	4	SOL, I	+20	-55	-63
						SOL, II	+15	-44	-44
						PL, I	=	=	=
						PL, II	=	-29	-40
105	R, F344.BN, ♂	6	HLS	14 d	6	SOL	-37*	-72	-42*
72	R-SD, ♀	3	HLS	14 d	4	SOL	-17	-55	-45
106	M-C57BL/6J,♂	2	HLS	14 d	?	SOL	=	-40[ww]	-
				28 d			=	=	
				42 d			=	=	
				56 d			=	=	
Space flight									
70	H, ♀, ♂	-	S	11 d	5	VL,I	=	=	=
						VL,II	-18	-47	-23
107	R-F344, ♂	adult	S	10 d	10	SOL, I	=	-23	=
						SOL, II	=	-12	=
76	R-SD, ♂	adult	S	14 d	5	SOL, I	-14*	-46*	-31*
						SOL, II	=	=	=
Tenotomy									
103	M-NMRI,♀	-	T	14 d	-	EDL	=	-18	-

*H: Human; R: Rat; M: Mouse; C: Cat; ♀: Female; ♂: Male; SD: rat strain Sprague-Dawley; W: rat strain Wistar; WI/HICK: rat strain WI/HICK; F344: rat strain Fischer 344; F344.BN: rat strain Fischer$_{344}$ x Brown Norway; B: Bed rest; DNV: denervation; SI: spinal cord injury; HLS: Hind-limb suspension; S: Space flight; T: Tenotomy; (N): number of animals; MN: myonuclear number; FCSA: fibre cross-sectional area; MD: myonuclear domain; Muscles: Diaph, Diaphragm; PL, Plantaris; EDL, Extensor digitorum longus; SM, Sternocleidomastoideus; SOL, Soleus; TA, Tibialis anterior; Gast, gastrocnemius; I, II(A,B) refer to muscle fibre type; All changes have been expressed as the change compared to a control normal muscles; =: no significant change between young and old animals; -: not reported; *: change estimated from authors graph; ww: % change in wet weight muscle mass.*

Table 2. Studies investigating the effect of atrophy on the myonuclear domain size.

cell depleted mice is realised by nuclei that are 'working flat out'. This may cause, admittedly speculative, nuclear dysfunction that over time may jeopardize the maintenance of the muscle fibres by attenuated protein turnover. It would therefore be interesting to know to what extent the quality of the muscle fibres of these hypertrophied muscles is affected over the life span of the animal. The addition of new myonuclei is a costly process and the fact that hypertrophy in normal organisms is associated with nuclear accretion is an indication of the importance of acquisition of new myonuclei for the maintenance of the increased muscle mass.

Overall, it appears that the nuclear domain is far from fixed, but rather increases during the development of hypertrophy (scenario in Figure 4C). However, there might be a 'ceiling' of the myonuclear domain size, or a maximal volume of cytoplasm that can be controlled by a single nucleus, beyond which hypertrophy can only proceed and be maintained for a prolonged time by addition of new myonuclei.

Myonuclear domain and decreases in muscle size

Skeletal muscle atrophy is largely, if not solely, attributable to a decrease in fibre size. According to the concept of the myonuclear domain this should be associated with a proportional decrease in the number of myonuclei to maintain the myonuclear domain size. Below we will discuss what happens during muscle atrophy resulting from disuse and ageing, while ignoring the loss of fibres contributing to the atrophy during ageing (a nonexistent fibre will have no myonuclei).

Myonuclei can be eliminated by a process called apoptosis. Originally, this term was given to the types of cell deaths that were executed by intrinsic 'suicide' instructions and characterised by cell shrinkage and fragmentation of cell organelles that are ultimately digested by rapid phagocytosis[64,65]. During apoptosis, a cell nucleus can be degraded without the immediate destruction of the cell. In skeletal muscle, the ultimate event of nuclear apoptosis is the fragmentation of myonuclear DNA executed by so-called caspases. The activity of these enzymes is regulated by complex signalling pathways that involve the interaction of different pro- and anti-apoptotic factors[66]. Unlike other cell types, nuclear apoptosis in muscle fibres is very rare under normal physiological conditions[67,68], but there is an increased expression of pro-apoptotic factors and number of apoptotic myonuclei following various atrophic stimuli[66,69]. The significance of nuclear apoptosis during atrophic conditions is, however, controversial.

Atrophy

The concept of a constant myonuclear domain size implies that muscle fibres loose myonuclei in proportion to (disuse-induced) atrophy. However, several observations do cast doubt on this concept in case of muscle atrophy. After 11 days of space flight, for instance, type II fibres in the vastus lateralis muscles from 2 out of 5 astronauts lost 18% of the myonuclei per mm fibre length, while the fibres atrophied by 47%[70]. This thus would result in a decreased, rather than constant, myonuclear domain size. Animal studies on the effect of disuse on the myonuclear number have generated some equivocal results (overview given in Table 2). In some studies a 50-70% reduction of the FCSA was accompanied with a considerable decrease in the number of myonuclei[52,71,72], whereas in other studies a similar magnitude of atrophy was not associated with any change in the myonuclear number[14,73-75]. Part of this discrepancy might be explicable by the different response of type I and type II fibres to disuse. In rat soleus muscle for instance, both myonuclear number and FCSA were reduced after 12-days spaceflight in type I but not in type II fibres[76]. This is not unequivocal, however, as in denervated gastrocnemius muscles type I, IIA and IIB fibres all atrophied to the same extent and at the same rate without loss of myonuclei[14]. Even in the studies that observed myonuclear loss, this was not proportional to the magnitude of atrophy, and hence myonuclear domain size decreases during atrophy (Table 2), even independent of fibre type[14]. The decrease in myonuclear domain size is not explicable by a decrease in fibre oxidative capacity as observed during e.g. denervation[77] which would have to be associated with an increased rather than decreased myonuclear domain size.

The maintenance of myonuclei may be an appropriate strategy as the breakdown of myonuclei is an energy requiring process. Maybe even more important is that the muscle is 'ready' to respond to stimuli that induce hypertrophy. Newly acquired myonuclei during hypertrophy in mice muscle are not lost after subsequent 3 months of denervation[78] and this might provide the muscle with a 'memory' for later re-growth. These and other observations, such as satellite cell proliferation[14,79-81], increased RNA and ribosomal concentration[14,82] and higher capillary density[77], during the early stages of (denervation-induced) atrophy suggest that while proteins may be broken down rapidly the machinery and structures required to rapidly regain muscle mass are at least to some extent preserved. This machinery may also be required in the initial stages of atrophy to cope with the dramatic changes in gene expression[83], and synthesis of enzymes and proteins involved in protein breakdown. This has indeed been observed after denervation where the expression of atrogens is transiently elevated 3-7 days after denervation[84-86] coinciding with the period of most dramatic atrophy[14,77]. During long-term atrophy, such as induced by denervation, the muscle regenerative capacity will ultimately be negatively affected[87]. These observations highlight a 'window of opportunity' where interventions to maintain muscle mass during hospitalisation and spaceflight are most effective early after the onset of disuse atrophy and may lose some of the effectiveness over time.

Ageing

The primary determinant of the age-related muscle weakening is the loss of muscle mass, also referred to as 'sarcopaenia' (see for review[88]). The process of sarcopaenia starts to manifest itself at the age of 60 and is the consequence of both a loss of muscle fibres and fibre atrophy[89]. If the concept of a constant myonuclear domain size would also apply to the ageing muscle, the age-related atrophy would be associated with a proportional reduction

Reference	Species	Age young (N)	Age old (N)	Muscle	change MN (%)	change FCSA (%)	change MD (%)
Increase in MN							
91	H, ♀	23±3 y (14)	76±4 y (14)	VL	19	-	-
	H, ♂	26±3 y (15)	74±4 y (13)	VL	19	-	-
92	H, ♂	20±1 y (8)	76±1 y (8)	VL, II	13	-27	-31
93	R-WI/Hick	4 mo. (-)	32 mo.	LA	39	-54	-80
13	R-F344	5 mo. (6)	24 mo. (8)	SOL, I	33	=	-24
				SOL, IIA	65	=	-34
14	R-W	5 mo. (15)	25 mo. (10)	Gast	36	37	=
99	H, ♂	27±2 (5)	82±3 (9)	VL, I	23	23	=
	H, ♀	28±2 (4)	84±8 (8)	VL, I	38	38	=
				VL, IIA	44*	-15	-41
No change in MN							
99	H, ♂	27±2 (5)	82±3 (9)	VL, IIA	=	-31	-33
41	H, ♀ ♂	22 (6) & 46 y (6)	65.1 y (6)	*Mixed*	=	=	-
94	H, ♀ ♂	24±4 y (4)	69±7 y (7)	Unknown	=	=	-
54	H, ♀	27.9±1.1 y (13)	62.8±1.0 y (13)	VL	=	-18	=
	H, ♀	26.1±1.4 y (13)	64.5±1.1 y (13)	VL	=	=	=
95	H, ♂	22.5±5.8 y (7)	65.0±6.0 y (8)	VL	=	-24	=
96	R-BN, ♂	5.5 mo (5)	27 mo (5)	TA	=	-8	
97	R-F344.BN, ♂	6 mo (6)	32 mo (6)	SOL	=	-27	-29
13	R-F344	5 mo (6)	24 mo (8)	PL I,IIA, IIB/D	=	=	=
33	R-W	5 mo (12)	25 mo (10)	PL	=	=	=
Decrease in MN							
28	M	12 mo (-)	26-29 mo	TA	-20*	-33	=
90	M, ♀	14 mo (-)	23 mo	EDL	-20	↓	=
				SOL	-16	↓	=

*H: Human; R: Rat; ♀: Female; ♂: Male; (N): number of participants / animals; RT: Resistance training; MN: myonuclear number; FCSA: fibre cross-sectional area; MD: myonuclear domain; Muscles: VL: vastus lateralis; PL: Plantaris; EDL: Extensor digitorum longus. All changes have been expressed as change in old compared to young muscles; =: no significant change between young and old animals; *:change estimated from authors graph.*

Table 3. Studies on the impact of ageing on the myonuclear domain size.

of the myonuclear number. Studies that have assessed the effect of ageing on the number of myonuclei are somewhat contradictory (see for an overview Table 3). The only two reports of a decrease in the number of myonuclei per unit muscle fibre length in old compared to young muscles were in mice[28,90], while in human, rat and bird skeletal muscles, the number of myonuclei remained unchanged or even increased during ageing. It is not clear what causes these discrepancies, but the two studies that reported an age-related decrease in myonuclear number[28,90] analysed isolated single fibres, whereas in all other studies myonuclear number was determined from muscle cross-sections. However, even studies using the 'cross-sectional' approach have come to contradictory results with both an increase[13,14,33,91-93] and no change[13,41,54,94-97] in myonuclear number during ageing having been reported. These discrepancies may be more apparent than real and when one considers also the changes in fibre size most of the discrepancies are readily explicable. For instance, in the four studies that did not observe any change in the number of myonuclei per muscle fibre during ageing there was also no or only a small change in the FCSA. Only in old women, a subgroup in the study by Petrella and colleagues[54], there was atrophy without a significant reduction in myonuclear number. In rat gastrocnemius muscle there may have been a loss of fibres between the age of 5 and 25 months which was compensated by an increase in size of the remaining fibres[14]. This increase in fibre size is accompanied with a proportional increase in the number of myonuclei, thus maintaining the myonuclear domain size[14], also in the plantaris muscle[33]. It thus seems that despite loss of muscle fibres during ageing, the relationship between fibre size and myonuclear number is maintained.

Another factor to consider is the absence of age-related muscle atrophy in some studies suggesting that ageing had not yet manifested in the muscles from the 'old' participants. In fact, several studies indicate that the age-related reduction in muscle volume and/or function occur mainly after the age of 70 (see for review[88]) and the mean age of the 'old' participants recruited in several studies[41,54,94,95] was below the age of 70 years. Similarly, in two[96,97] out of the three studies that reported

no change in the number of myonuclei per muscle fibre for the aged rat muscle, the age of the 'old' rats was still 4-5 months below the mean life expectancy of their strain and not really old animals[98].

At very old age (i.e. >70% of the maximal live expectancy) the nuclear domain size may decrease, something that has indeed been observed in muscles from very old (32-month-old) rats[93]. Although in humans an increased number of myonuclei per fibre have been observed at advanced age[91], this did not necessarily result in a decreased myonuclear domain size[99]. What is interesting, however, is that the distribution of myonuclei that is thought to be such that it minimizes diffusion distances between nuclei[27], becomes more heterogeneous during ageing[90,99] and may impair local protein turnover.

Overall, the pattern emerges that up to a certain age the number of myonuclei per muscle fibre are not substantially affected by ageing even when muscle fibre atrophy has started. Further it seems that during ageing the number of myonuclei per muscle fibre may even increase. For humans, this may be after 70 years of age, while in rats this age may (somewhat) depend on the strain. As a result of the atrophy and increase in myonuclear number, myonuclear domain size may decrease at advanced age.

Concluding remarks

The short answer to the question addressed in this review is that the myonuclear domain size is not fixed. Although one might then suggest abandoning the concept of a constant myonuclear domain entirely, this is too radical as in the normal situation a relationship between fibre size and myonuclear number does exit. It is only during maturational growth, ageing and when the muscle adapts to altered functional demands that deviations occur. Deviations might be understood when one realises that: 1) addition and removal of myonuclei is an expensive process and 2) a nucleus needs a reserve capacity to be able to respond to sudden changes in transcriptional demands and 3) during hypertrophy it must be able to bridge the period between activation and incorporation of satellite cells into the myofibre. The preservation of myonuclei during atrophy may be an excellent strategy to save energy and at the same time have the muscle 'ready' for subsequent recovery of muscle mass. Another factor that might be of importance, as we suggested before[100], is the distribution of myonuclei within the muscle fibre. While a lot of attention has been given to the size of the myonuclear domain in terms of cytoplasmic volume, more attention may need to be given to the number per fibre perimeter and distribution of the myonuclei over the perimeter of the fibre, given that during for instance maturational growth the number of myonuclei per fibre radius remains constant[41].

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