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Review Article

What determines myonuclear domain size ?

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Abstract

The muscle cell is multinuclear and each nucleus controls transcriptional activity in the surrounding territory of cytoplasm called myonuclear domain (MND). MND size varies with the fiber type and is inversely proportional to the muscle fiber oxidative capacity. Change in MND size precedes change in myonuclei count during post-natal growth and most conditions of muscle fiber hypertrophy, suggesting that the myonuclei have the ability to enhance their synthetic capacity according to cell size, functional and metabolic needs. MND size has a "ceiling" limit during hypertrophic process beyond which extra myonuclei are donated by satellite cell to support further muscle growth. During ageing-related atrophy, myonuclei are not lost but an unequal distribution is reported. Ageing myonucleus still responds to resistant exercise and hormone replacement therapy (HRT) by enhancing its transcriptional capacity. Thus the MND size is far from constant and modulates itself to contribute to the muscle remodeling in various conditions.

Introduction

Skeletal muscle is a highly plastic organ that can adapt itself in response to altered activity. These adjustments in response to functional and metabolic demands elicit qualitative and quantitative changes in protein metabolism and gene expression that can result in change in size, functional and metabolic properties of muscle fibers. A single muscle cell contains hundreds of nuclei each of which controls transcriptional activity in its cytoplasmic domain called myonuclear domain (MND) (1-2). The number of myonuclei in a muscle fiber and transcriptional activity per myonucleus are not constant (3-4) and are two prime regulators of muscle fiber protein synthesis and thus, the cross-sectional area (CSA). Since fiber size is directly proportional to the force generating capacity under most conditions, so fiber hypertrophy and atrophy are the key determinants of changes in muscle strength in health and disease. A constant MND size has been proposed during change in fiber size so that hypertrophy is accompanied by incorporation of new nuclei via satellite cells (Fig. 1B) while atrophy is related to nuclei loss via apoptosis (Fig. 1C) (5). However, a good correlation between fiber CSA and myonuclei count is not always reported (6-9). For instance, hypertrophy induced by overload precedes myonuclei incorporation (10). Similarly, MND size has been reported to increase with maturational growth and decrease during ageing (9). Denervation-induced atrophy was not related to loss of myonuclei in mouse plantaris (7) and rat diaphragm (11) muscle.

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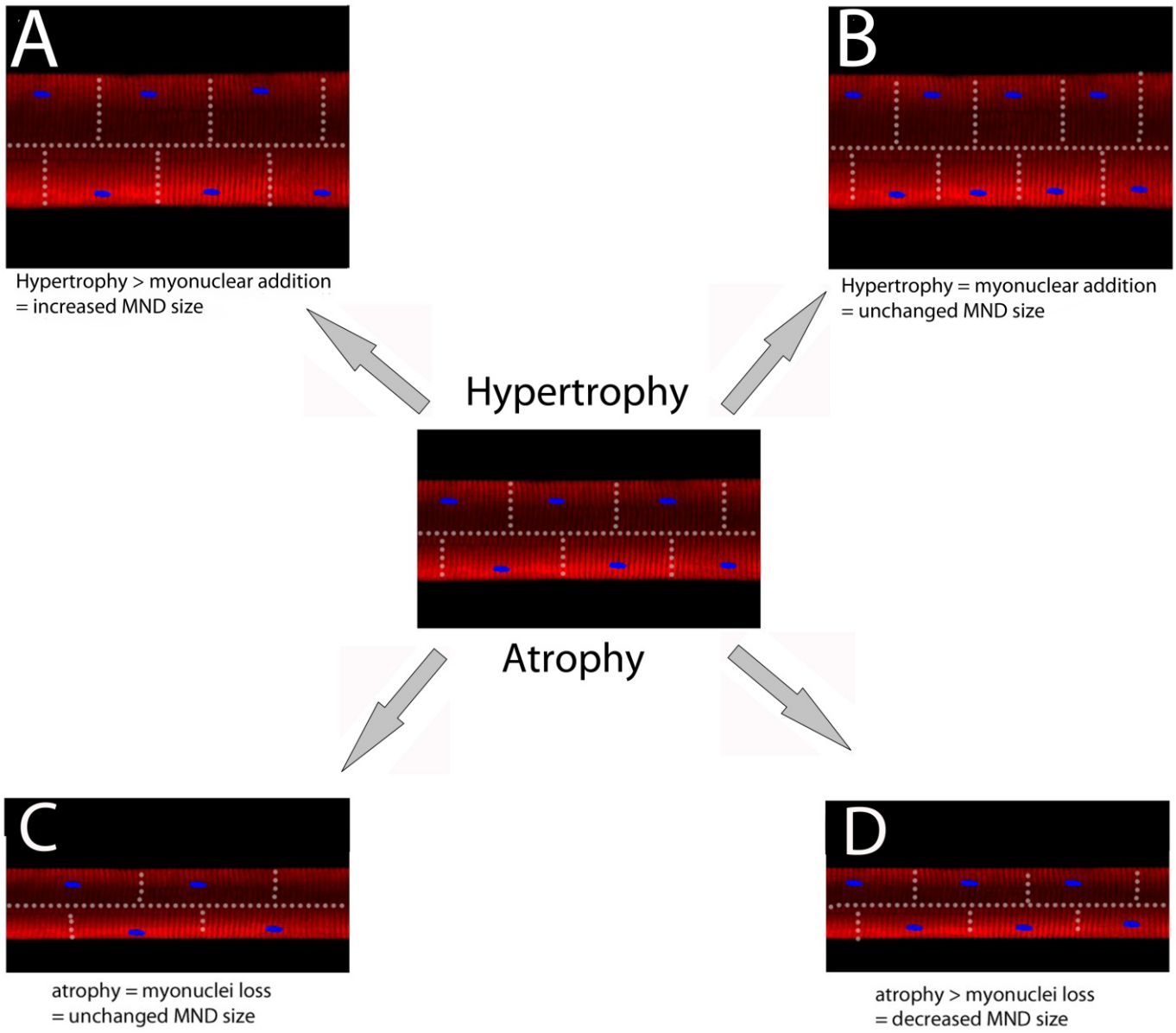

Fig. 1: Possible changes in myonuclear domain (MND) size with muscle fiber hypertrophy and atrophy.

Most of the studies for MND size measurement have been performed on frozen sections from muscles, where freezing and stretching procedures can introduce variability in fiber sarcomere length and CSA (12). Further, altered shape and elongation of myonuclei may result in over-counting of nuclei from cross-sections, especially in old age (13). Also, it is difficult to distinguish myonuclei from satellite cells without using electron microscope and all these factors may lead to erroneous measurements of MND size and myonuclei count in muscle sections. Such problems can be avoided by measuring MND size at single fiber level at a fixed sarcomere length for optimal force generation.

Single fiber model also gives us an opportunity to study positioning of nuclei in three-dimensions which is important for optimal transport distances across the fiber, since not all the nuclei are transcriptionally active for all the at any given time point (14-15). Modeling studies from mouse single fibers indicate that the nuclei are not randomly distributed but more

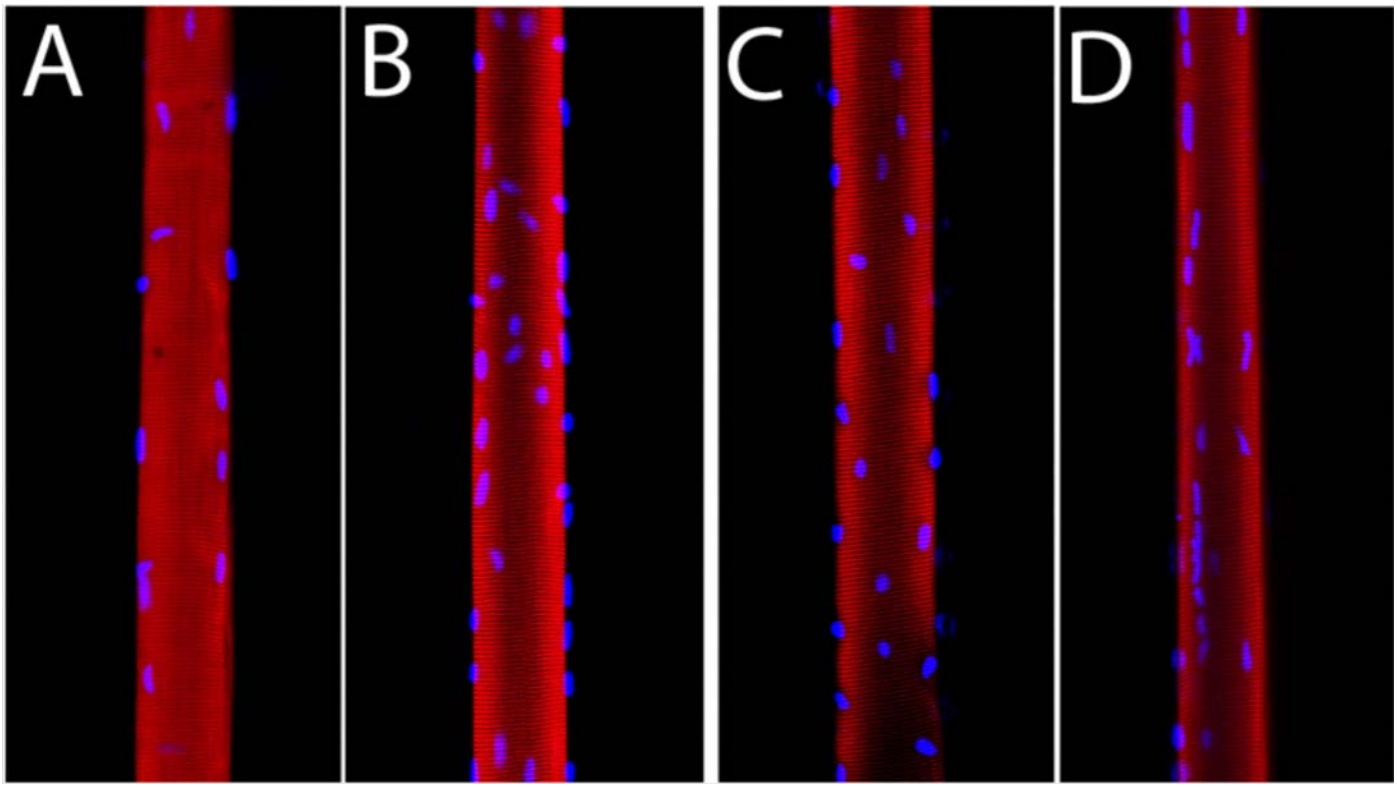

Fig. 2: Confocal microscopy images of single muscle fibers from mouse EDL (A) and soleus (B) muscles and from young (C) and old (D) human subjects. In mice myonuclei are more orderly placed in the EDL (A) while distribution is less ordered in the soleus (B) muscle fibers. In humans, the regular distribution of myonuclei observed in young age (C) is lost is old individuals (D). DAPI (blue) stained myonuclei while the rhodamine phalloidin (red) stained actin. Scale bare = 50 μm.

or less evenly placed to minimize the transport distances (16). The ordered pattern is more prominent in fast- than slow-twitch muscle fibers (Fig. 2A-B). Also, the representative nuclei from two fiber types are different in their phenotype in rodents. While the myonuclei from fast-twitch fibers are predominantly elliptical and aligned to long axis of the fiber, nuclei from slow-twitch fibers have more rounded appearance. Although some of the nuclei have shapes varying between the perfectly round and perfectly elliptical scheme (Fig. 3) (17). These differences are bit arbitrary in human muscle fibers.

The general purpose of this review is to relate the MND size with the remodeling of skeletal muscle in response to change in cell size, functional capacity and MyHC isoform type.

Effects of fiber type and mitochondrial contents

Myonuclear domain size appears to differ between fibers expressing different MyHC isoforms i.e. slow-twitch fibers have smaller MND size than fibers expressing fast-twitch MyHC isoforms (Fig. 4) (5, 8). This is probably a consequence of higher protein turnover rate and shorter half-life of slow *vs.* fast MyHC isoform (18). Fibers expressing type I MyHC isoform are highly active in protein synthesis, hence a demand for higher mRNA transcription and a greater concentration of myonuclei. Further, MND size is inversely correlated to the muscle fiber oxidative capacity and mitochondrial contents (19). Metabolic demands and the mitochondrial volume density of muscle fibers are higher in smaller mammals and decrease with increasing body size (20-21). There is a dependency on the fiber type too. For instance, in human muscle, mitochondrial density is higher in fibers expressing type I MyHC isoform followed by type IIa and IIx fibers (22). However, this pattern is not observed in small mammals such as rodents, where the muscle fibers expressing the type IIa MyHC isoform have higher mitochondrial density than the

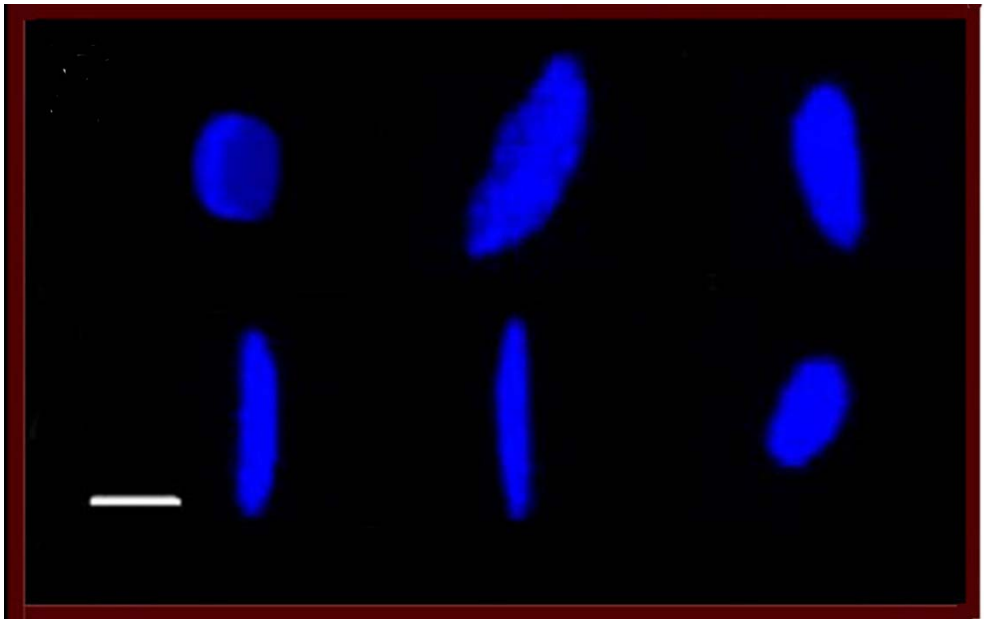

Fig. 3: Confocal microscopy images of myonuclei with various shapes. Scale bar = 3 µm.

MyHC Isoform type	Cross-sectional area	Protein turnover rate	Mitochondrial density	Endurance	Myonuclear count	MND size
Slow-twitch (Oxidative)	Small	High	High	High	High	Small
Fast-twitch (Glycolytic)	Large	Low	Low	Low	Low	Large

Fig. 4: The properties of the slow- and fast-twitch fiber types. There are exceptions to this general design. MyHC = Myosin heavy chain.

type I fibers (23-24). We have related this with slightly smaller or equal MND size in type IIa fibers of rodents compared with type I fibers (17, 25), suggesting that the mitochondrial proteins beside the MyHC isoform type play a role in determining MND size. This notion is further supported by a recent finding that the MND size is higher in the type IIa fibers from superficial glycolytic region of rat plantaris muscle than the type IIa fibers from deep oxidative region (26). However, we have recently observed in myostatin knock-out mice that the MND size was larger in type IIa fibers than in type I fibers although the SDH staining was deeper in type IIa fibers in series cross-sections (17). This suggests that even the mitochondrial proteins may not be the prime determinant of MND size at least in myostatin knock-out mice.

MND size and Hypertrophy

The skeletal muscle fiber hypertrophy is characterized by an increase in the size and number of myofibrils (27). This is achieved by an enhancement in the muscle fiber transcriptional

activity and protein synthesis (28-29) and the ordered assembly of newly formed sarcomeric proteins (30-31). Newly formed nuclei donated by satellite cells, at least partly, contribute to the increase in transcriptional capacity (29), as indicated by hypertrophy of the skeletal muscle linked with myonuclear accretion in rat (32), cat (33) and humans (34). This is supported by attenuation (35-36) or prevention (27, 37) of hypertrophy by 'gamma irradiation' which blocks satellite cell activity. However, hypertrophy independent of satellite cell activation has also been reported (38-40). While much of the emphasis has been given to the nature and duration of exposure of the hypertrophic stimuli, muscle specific response should also be considered since satellite cells from fast- and slow-twitch muscles have difference in proliferation and differentiation potentials (41). We have recently shown that the myofibers from fast- and slow-twitch muscles respond differently in terms of their MND size to the same hypertrophic stimulus (17). The muscle fibers from fast-twitch EDL have lower number of nuclei to begin with, which are optimally tuned for force generation. Hence, the addition of new myonuclei may or may not occur in response to hypertrophic stimulus depending upon functional outcome. On the other hand, myonuclei from slow-twitch soleus muscle fibers can expand their domains in response to myostatin deficiency or IGF-1 over-expression without the need for extra myonuclei. This shows a greater dynamic range of cytoplasmic expansion and transport distances in slow- than fast-twitch fibers. It must be noted that, other hypertrophic stimuli such as overload can lead to satellite cell activation in soleus muscle (38, 42-43). Such differences in hypertrophic response may be attributed to different signaling pathways leading to hypertrophy. For instance, calcineurin induces muscle fiber hypertrophy via its affect on satellite cells stimulation (44-45) and fusion (46). On the other hand, P13K/Akt pathway spares the satellite cells (40) and instead, activates anabolic pathway *via* the mTOR signaling and inhibits catabolic pathway *via* the FOXO transcription factors (47).

Satellite cells are responsible for myonuclear addition during post-natal muscle growth (48-50). In addition, their proliferation can be evoked following acute injury (51) and in response to muscle overuse and increased tension (52). During hypertrophy, satellite cell proliferation and myonuclear addition lag behind myofiber growth suggesting that the initial phase of hypertrophy is characterized by enhanced transcription per myonucleus (53) leading to a slight expansion of existing MNDs (Fig. 1A) which may persist up to four weeks (26). This is followed by fusion of new myonuclei thus re-establishing the muscle fiber DNA to cytoplasmic volume ratio (Fig. 1B) (32, 43). This phenomenon also supports the concept of MND ''ceiling'' (17, 34) discussed later in this review.

MND size and post-natal muscle growth

Early post-natal muscle growth has been linked with myonulcear accretion as shown by an increase in DNA content (54) and myonuclei count (55-56) in maturing myofibers. New nuclei are provided by the satellite cells via the proliferation and fusion to the maturing myofiber. Thus when satellite cells proliferation is blocked by gamma irradiation (57) or by hind limb suspension (58) in growth phase, the normal increase in myonuclei count and fiber CSA during post-natal muscle growth is attenuated. The number of satellite cells decreases steadily during normal growth in rats (59-61) and humans (62-63). For instance, human satellite cells constitute 15% of all myonuclei at birth, 6-10% at two years of age, 4% in adults and less than 2% in old age (64). The decline in number, along with rapid loss of proliferative capacity of satellite cells with increasing age (65) explains why the myonuclear incorporation slows down with maturational muscle growth.

The number of myonuclei can be coupled with increase in fiber CSA in early growth phase (16). However, the increase in myonuclei number lags behind increase in fiber size, resulting in expansion of MNDs in early post-natal growth. Using single fibers from mouse EDL, (50) reported a ~5.7 fold increase in MND size from P7 to P56 which is consistent with findings from rat diaphragm (49) and soleus muscle fibers (48). Further, while the protein turnover rate is lower in fast *vs.* slow-twitch muscle fibers (66), a higher level of protein degradation is described in young than middle age mice (67-68).

This shows that the myonucleus in early growth phase probably has higher transcriptional activity than the ageing myonucleus, to overcome both the higher protein degradation and expanding cytoplasmic domain.

MND size and Sarcopenia

Ageing is associated with loss of muscle mass, also referred to as sarcopenia (69) which is characterized by both a decrease in muscle fiber number and size. Further, a reduced adaptability of myonuclei is also reported as shown by decline in satellite cells density (4) and proliferation capacity (70). Old age is also associated with higher level of protein degradation in muscles (67-68) which might require greater amount of genetic machinery to synthesize proteins. The number of myonuclei and the size of MND would be critical if the quality of myonuclei is compromised as suggested by lower translational capacity in old age (3, 71). An increased (4) or unchanged (72) myonuclei number has been reported with ageing in human muscle. The same observation of an increased (10, 73) or unchanged (74-75) myonuclei count has been reported in old rats as well. These discrepancies may be partly explained by the nuclear aggregate formation and altered morphology observed in ageing muscle fibers (Fig. 1D) (13, 16, 76). Further, information from human muscle cross-sections were not corrected for fiber-types (4, 77) which may give rise to erroneous conclusions since slow-twitch muscle fibers have smaller MND size than fast-twitch fibers (5, 8) along with an ageing-related fiber-type transition (78) and a preferential atrophy of type II fibers (79).

We have reported in the human single muscle fibers that the myonuclei respond to ageing process in a fiber-type specific manner (76). While the average MND size in type I fibers was not affected by ageing, a significant decrease in MND size was observed in type IIa fibers when compared to young individuals. Such a decrease in type IIa fiber MND size is consistent with previous findings (13) and may reflect a decrease in myonuclei efficiency to govern a smaller territory of cytoplasm in atrophied muscle fiber. Addition of more myonuclei may be a further attempt to rectify the inefficiency and to keep the transport distances in check for ageing myonucleus. On the other hand, type I fibers show an increase in the CSA and myonuclei count, in agreement with the weak correlation between size and nuclei count reported in old age (16). An interesting finding in the old age is the altered spatial distribution of myonuclei discussed later in this review.

MND size and therapies for sarcopenia

Resistant exercise and food intake

Physical activity especially resistant exercise has been acknowledged as a potent natural stimulus to promote muscle protein anabolism in elderly (80-82). Current theories also suggest ingestion of amino acids and/or proteins (83) since protein turnover rate in skeletal muscle is highly responsive to nutrient intake (84).

Most training studies in elderly do not report myonuclear addition in response to resistant training, thereby suggesting an expansion of the existing MNDs (34, 85). This is in contrast to young people, where exercise leads to both an increase in fiber CSA and myonuclei count (34, 86-87). Thus, the aging muscle responds to resistant exercise by primarily increasing protein accretion rather than myonuclear incorporation, mainly attributed to an increase in myofibrillar protein synthesis (88-89). The lack of myonuclear addition may be explained by small number (90) and proliferative capacity (65) of satellite cells in elderly. Also, satellite cells become less responsive to mechanical overload with ageing in animal models (75, 91-92). Thus, the exercise-induced senile muscle primarily relies on increased protein accretion rather than increased genetic factories to combat ageing-related muscle loss. This shows that the muscle protein synthetic machinery maintains the ability to respond to the anabolic stimuli such as exercise and protein intake, up to old age. Recently it was shown that the senile muscle maintains a sort of ''memory'' from young age where the nuclei obtained in young age via overload hypertrophy are not lost during atrophy or ageing process (93). The author suggests that such a ''filling up'' of young muscle with nuclei by exercise may prove beneficial in old age by facilitating re-growth

despite long period of inactivity in between. More research is required to look into interaction of exercise, nutrition and aging on to the myonuclei, MND size and especially the myonuclear spatial organization, since it is the altered spatial distribution and not the loss of myonuclei, suggested to be more important biological finding than change in MND size in old age (76).

Hormone Replacement Therapy (HRT) in post-menopausal women

HRT is used by post-menopausal women to counter menopause related loss of muscle mass and function (94-95). Most recent studies favor beneficial effects of HRT on skeletal muscle (96-98). Estrogen is known to augment satellite cells activation and proliferation in post-exercise muscle (99) through estrogen-receptors mediated mechanisms (100). Despite this, less is known about effects of estrogen on myonuclei and MND size in skeletal muscle fibers.

Recently, we examined the effects of HRT on myonuclei in single muscle fibers from post-menopausal twins discordant for hormone replacement therapy (101). We report that the effect on myonuclei is fiber-type specific. While myonuclei in slow-twitch type I fibers rearrange themselves leading to smaller MNDs, no effect of HRT was observed on the mean MND size in the fibers expressing the type IIa MyHC isoform. This disagreement may be explained by estrogen's anti-oxidant properties (102) and a higher concentration of its receptors in slow- than fast-twitch fibers (94, 103). Slow-twitch fibers are also transcriptionally more active (104) and more susceptible to ageing-related oxidative damage (105) than the fast-twitch fibers. Estrogen accordingly may reduce MND size and arrest oxidative damage in slow-twitch fibers to optimize function and cytosolic transport distances. These findings may help us devising new strategies to combat sarcopenia in post-menopausal women.

In the concluding section, we try to discuss a couple of interesting questions regarding MND size.

Is there a MND ceiling ?

The myonucleus is probably not working at its maximum synthetic capacity normally and has the ability to augment its capacity in response to change in fiber/MND size. A single bout of resistant exercise can lead to increased protein synthesis for ~24 hours (106). Further, the initial phase of myofiber hypertrophy is characterized by enhanced transcription and translation (53) without addition of new myonuclei, leading to slight expansion of existing myonuclear domains. Thus, the existing myonuclei can support the hypertrophy as long as a certain "threshold" for transcriptional activity is not reached (77, 107). Beyond the threshold limit, further hypertrophy is supported by fusion of new myonuclei donated by satellite cells (43). For instance, the hypertrophy beyond ~26% is supported by myonuclear accretion (32, 87) but not up to ~15% (108). Using a cluster analysis of 16 weeks resistant training program on humans (34), a theoretical MND ceiling size of ~2000 μm^2 was proposed that can be attained with enhanced protein synthesis before myonuclear accretion becomes mandatory to support further hypertrophy. However, this value is derived from muscle cross-sections and does not take into account fiber type variations although MND values vary considerably with fiber types (5, 19, 32).

Recently, using the mice single muscle fibers we have extended the concept of MND ceiling size to the hypertrophy with or without functional compromise (17). Thus, the myonuclei from hypertrophic fast-twitch EDL fibers can expand their domains by ~10% without compromising force-generating capacity. Any further enlargement of MNDs will result in decrement of force although hypertrophy may still occur. Based on these findings, we suggest a MND ceiling size of ~32,000 μm^3 beyond which myonuclear accretion is a prerequisite for functional hypertrophy. We also show that nuclei from slow-twitch muscle fibers have a bigger dynamic range for domain size without compromising force or hypertrophy. This shows that the MND size can be coupled with the force generating capacity in single muscle fibers. We have shown before that the MND size scales with body mass in a variety of mammals (25) which probably mean that the theoretical MND ceiling size may vary between species.

Does distribution matter ?

While much of the emphasis has been given to the mean MND size in muscle adaptation process, more attention needs to be given to the distribution across the cell surface since for instance the mean myonuclei count may not vary between young and old age mice (16). Reports from single fibers indicate that the myonuclei are arranged in a regular positioning across the muscle fiber perimeter, more so in fast-twitch EDL than slow-twitch soleus muscle fibers. (8). Such a pattern of nuclear positioning may facilitate inter-nuclear communication for regulation and coordination of protein expressions. The significance of nuclear positioning can be understood by the finding that not all the nuclei are active for all the genes (14) and at the same time (15). Many factors have a role in determining nuclei positioning such as desmin and blood vessels (109) and microtubuli (16). Mice deficient in desmin show irregularities in placing of nuclei in the muscle fibers from thigh (110) and EDL muscle (111). Further, microtubules organization is altered by denervation, leading to nuclei cluster formation (112). When a denervated muscle is chronically stimulated for two weeks, an increase in vascularization is observed along with myonuclei positioning along the newly formed blood vessels in muscle fibers (113).

Recently, we reported an ageing-related altered spatial organization of myonuclei in human single muscle fibers (Fig. 2D) (76) which is consistent with findings in mice (16). The denervation-reinnervation process going on in aging skeletal muscle (114) may play a role in it since long term denervation is associated with altered microtubules organization (112) and nuclei aggregate formation (115). Such a pattern of nuclear positioning may impair local protein turnover in the bare areas of cytoplasm where MND size is large, leading to increased chances of post-translational modification and compromised function of proteins.

In summary we believe that myonuclear domain size is far from fixed and can vary in the muscle adaptation process with size, metabolic and functional demands of the muscle. Muscle nuclei have a remarkable range of transcriptional capacity but a “ceiling” exists before the addition of new nuclei is required to further sustain myofiber hypertrophy. Such a ceiling value may differ for hypertrophy with or without functional compromise. Also, more importance should be given to the qualitative distribution and not just size of MNDs given that the size may stay constant, for instance during ageing process. A better understanding of the topic will help us formulate future pharmacological interventions focusing on protein metabolism and stem cell therapy in the ageing and atrophied muscles to improve quality of life.

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