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Myonuclear domain in skeletal muscle fibers. A critical review.

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The muscle cells, or myofibers, are one of the three multinucleated cell types, along with osteoclasts and cytotrophoblasts. This aspect of skeletal muscle has led to the concept of myonuclear domain, which is defined as the theoretical amount of cytoplasm within a muscle fiber controlled by a single myonucleus. Considering the huge plasticity of skeletal muscle fibers, the myonuclear domain has become one aspect of major interest under situations leading to muscle atrophy (spinal cord isolation and transaction, microgravity, hindlimb suspension and chronic denervation) hypertrophy (synergist ablation and bouts of exercise) and day-to-day wear and tear (age, body size, muscle function and fiber type-related differences). This concept is supported by several studies reporting that changes in muscle fibers' cross-sectional area (CSA) are paralleled by an alteration in the number of myonuclei, maintaining constant the relationship between them. This implies a fairly strict regulation of the myonuclear number that is governed by two opposing mechanisms: the gain of myonuclei by the fusion of muscle stem cells into hypertrophying muscle fibers and the loss of myonuclei by apoptosis/necrosis in atrophying muscle fibers. The aim of this review is to scrutinize the changes in the myonuclear domain in skeletal muscle fibers considering the atrophic and hypertrophic processes, emphasizing the differences between the various methods utilized to achieve these morphological alterations, and the differences between muscle responses according to their phenotype (different function and fiber type composition) and animal age (relevance to sarcopenia).

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1. INTRODUCTION

After the discovery that the skeletal muscle fiber is a multinucleated structure derived from the fusion of myoblasts forming a syncitium where different nuclei share their own products (14), the concept of myonuclear domain (MND) has sprouted. This idea brought to light the theoretical concept that a single myonucleus "supports" a determined amount of cytoplasm in order to grant the functionality of the entire fibre (14), i.e., the MND is the theoretical volume of cytoplasm within the myofiber regulated by the gene products of a single myonucleus (32).

One of the most significant studies related to this concept was made by Pavlath et al. (32). Their experiments demonstrated that the products of the several myonuclei in the skeletal muscle cell – proteins associated with the Golgi and the contractile apparatus – remain localized near their myonucleus of origin (32). Although some studies have reported that some surface membrane proteins (e.g., CD8) may be distributed over the entire muscle cell, it seems that the protein synthesis, processing and distribution occur in the vicinity of the correspondent myonucleus (14). However, given the possibility that the cytoplasmic or membrane proteins are free to diffuse, if they associate with a local target, they remain near the myonucleus; otherwise, they will occupy the entire length of the fiber (14). Therefore, their distribution will be determined by changes in the physiological or developmental state of the cell such as relative rates of diffusion, association with a target and metabolic breakdown (12, 14).

It is speculated that the MND tends to be relatively constant when different experimental protocols are applied to produce changes in the skeletal muscle size (3). In fact, different studies have suggested that the myonuclei number in the myofiber play an important role during the skeletal muscle size adaptation, since during muscle hypertrophy the myonuclei number are

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increased (probably through fusion of myogenic cells) while during muscle atrophy the total myonuclei number within muscle fibers are significantly reduced, hypothetically through apoptosis/necrosis (3).

Several studies investigating skeletal muscle hypertrophy suggest that the myonuclei number increases during the growth and enlargement of the muscle fiber (2, 13, 18, 27, 35, 37). In opposition, studies analysing the skeletal muscle atrophy process reported a proportional reduction between the myonuclei number and the fiber size (4, 16, 28, 31, 40). Nevertheless, different results have been reported. Concerning the skeletal muscle hypertrophy process, one study, using isolated fibers from mice, reported that the cytoplasmic enlargement during muscle hypertrophy is followed by a decrease in the myonuclear number (45). Regarding the skeletal muscle atrophy process, there are various studies reporting that the myonuclear number is not synchronized with the myofiber volume; i.e., during the reduction of the skeletal muscle mass the myonuclei number did not decrease (1, 11, 20, 21, 31, 39, 42, 44, 47). In a study where atrophy was induced by 28 days of hindlimb suspension (HS), a significant decrease in CSA and an increase in myonuclei density were found (20). Likewise, in a recent in vivo study (11) the results of different atrophy-inducing methodologies (denervation, nerve impulse block and tenotomy for mechanical unloading) essentially demonstrated the same conclusion – skeletal muscle atrophy was not followed by the loss of myonuclei. Some methodological imperfections and the fact that most studies focus on muscle undergoing changes (phases of muscular dynamic adaption) may be the main reason for the occurrence of these disparate results and, therefore, for the generated controversy regarding the relationship between skeletal muscle fiber size and its myonuclei number. Whether the gain or loss of fibers´ myonuclei is the major contributing factor to muscle fiber hypertrophy (gain) or atrophy (loss) remains the central controversial point.

Since there are several studies reporting conflicting results, a general agreement about this issue seems far from being achieved. However, the controversial points in the literature are those who incite curiosity and, therefore, are objects of study and investigation. Consequently, the main reason for this review is to gather and examine in detail the most relevant studies related to MND. The methodological procedures will be analysed in detail to determine what is responsible for the attainment of these incongruent results. Another aim of this review is to highlight the behaviour of MND according to the muscle fiber phenotype. Indeed, several studies described differences in the MND size according to the fiber type composition suggesting that the MND in the fast-twitch fibers is larger when compared to slow-twitch fibers (2, 41, 43). The MND regarding the subjects´ age and body size will also be addressed in the fourth chapter. Although the MND seems to be directly correlated to body size (23), this relationship is less clear when compared to the subjects' age (10, 21).

2. THE MND DURING THE SKELETAL MUSCLE ATROPHY PROCESS

The concept of MND in atrophying muscles has been tested in several experimental conditions, such as hindlimb suspension (HS) (1, 19-21, 42), space-flight (4, 16, 19), spinal cord transaction (SCT) (2, 38, 47), denervation (5, 11, 46), mechanical ventilation (28) and bed rest (31). It is known that the atrophy process is characterized by an increased muscle mass wasting and therefore a decrease in the myofibers' cross-sectional area (CSA) along with the disappearance of fibers´ myonuclei (3). Therefore, during atrophy, as the transcriptional and translational demands placed on myonuclei are attenuated, myofibers may respond by eliminating myonuclei, possibly to maintain a constant MND (8). However, the literature concerning this issue is filled with disparate results.

2.1. Hindlimb suspension

Several research groups using HS-induced atrophy have verified that the MND decreases during the atrophy process (1, 19-21, 42).

The decrement in the MND during muscle atrophy seems to be the consequence of a quantitative loss of the muscle fibers' CSA that is not always proportional to the decrease of the fibers' myonuclei number; i.e., although the myonuclei number decreases, this reduction is not sufficient to preserve the MND (1, 21).

One study analysing the soleus muscle of rats (6 months old) over 14 days of HS reported that the myonuclei number decreased along with the muscle fiber CSA (21). Nevertheless, the CSA per myonucleus ratio was also reduced when compared to the control group. The mean CSA was determined from 150 muscle fibers and the myonuclei number from 120-150 fibers (21). It remains unclear if the myonuclei number were counted in the same 150 fibers analysed for the CSA. Another study analysing the soleus muscle during two weeks of HS also reported that the atrophy process resulted in muscle CSA reduction, loss of myonuclei number and a decrease in the cytoplasmic volume per myonucleus ratio, although a significant correlation between the mean myonuclear number and CSA was found (1). The methodological procedure for the assessment of the myonuclei number per mm of fiber, muscle fiber CSA and volume, also used in other studies (2, 4), is

full of particularities. Using a confocal microscope, the myonuclei number average in isolated fibers was calculated from projections obtained from scans in a chosen portion of the fiber in a determined field (field size≈173 x 173 µm); fiber CSA was calculated from the reconstruction of serial scans and the volume per myonucleus was measured by multiplying the calculated CSA by the region length (173 µm) and dividing by the myonuclei number per field. To calculate the final results for each fiber (single value), three separate regions were arbitrarily picked, examined and averaged. All the fiber ends with damaged regions and connective tissue were excluded. To minimize the differences in the fibers´ state of stretch, the values for the myonuclei per mm and the fiber CSA were multiplied by the mean fiber sarcomere length (mean value was calculated in a field of 173 µm, from three different sets of 10 consecutive sarcomeres) and were divided by 2.5 to normalize to a 2.5 µm sarcomere length. Five to 15 fiber segments per animal were analysed (1, 2, 4).

Surprisingly, various studies have reported that this CSA per myoncleus ratio imbalance also can be the result of a preservation or increase of the myonuclei number during the atrophy process (19, 20, 42). One study inducing 28 days of HS in the plantaris and soleus muscles of rats and analysing the type of myosin expressed (fast and slow), demonstrated that the muscle CSA decreased in both soleus (both fast and slow fibers) and plantaris muscles (only in the fast fibers) (20). Interestingly, the myonuclei number per mm of fiber increased in the soleus muscle (fast and slow fibers) and was maintained in both fibers of the plantaris muscle. Regarding the cytoplasmic volume per myonucleus ratio, the results showed that only the slow fiber group of the plantaris muscle remained unchanged from the control fibers. To determine the myonuclei number per fiber the authors analysed four points (each point with 350 µm2) along the fiber segment. The mean value of the four points was taken as the mean myonuclei number per mm of fiber (assuming that the myonuclei density along the fiber is uniform). The muscle CSA was determined from the same four points analysed to verify the myonuclei number (careful attention was taken to minify any distortion or compression of the fiber) and the cytoplasmic volume per myonucleus was calculated multiplying the mean CSA of each fiber per 1000 (103 µm3) and, then dividing it by the myonuclei per mm. The length of each fiber was standardized to 2.5 µm per sarcomere. The total number of fibers analysed in the fast fibers of the plantaris muscle was 39 (16 from control group and 23 from HS group) and in the slow fibers was nine (three from control group and six from HS group). Likewise, the total number of soleus fast fibers analysed was eight (two from control group and six from HS group) against 49 slow ones (22 from control group and 27 from HS group) (20). This study has some methodological limitations that may compromise the obtained results. Apart from the reduced fibers sample size that does not allow a secure extrapolation to the real variability in the entire muscle, the methodological procedure used does not guarantee that the satellite cells were not erroneously counted as myonucleus.

The tibialis anterior (TA) and the gastrocnemius (Gast) muscles of juvenile rats were also analysed using this atrophy-inducing mechanism (19). A span of 5.4 days of HS resulted in a reduction of the fiber CSA in both muscles and in the maintenance of the myonuclei number per mm of fiber. The cytoplasm volume per myonucleus decreased in both muscles. These results were obtained from the analyses of three fibers from the TA muscle and three fibers from the Gast muscle (19). The methodological procedures used to determine the main variables in this study were those used in the former (20). Once again, the sample size seems to be insufficient to safely extrapolate these results for the whole muscle.

In a recent paper (42), the soleus muscle of rats was also submitted to inactivity by HS over 14 days. This process resulted in a decreased muscle CSA (50 percent in the slow fibers and 35 percent in the fast ones), a constant number of myonucleus per mm of fiber and decreased CSA per myonucleus ratio (36 percent from control group). The muscle CSA was determined by measuring 100 fibers, and no less than 200 fibers were analysed for the myonuclei number quantification (dystrophin stained). The mean values for both variables were calculated. The number of myonuclei per mm was calculated by the formula X= NL/(1+d) – where X is the myonuclei number per mm and N is the myonuclei number at its cross-section, L is the length of segment (1 mm), l is the section thickness and d is the myonucleus length (13.4 µm) (42).

All the studies mentioned reported that the cytoplasm (CSA or volume) per myonucleus ratio decreases. Nevertheless, the methodology used to determine this relationship may induce errors. These inaccuracies may be due to: 1) the use of equations to extrapolate the cytoplasm volume and myonuclei number per fiber; 2) the normalization of the fiber volume and length (the normal size of the fiber may be altered with the isolation process); 3) the characterization of an entire fiber only with the examination of four points along the fiber; and 4) the small quantity of fibers analysed.

2.2. Space flight

Space flight is another model used to produce chronic unloading in the skeletal muscle fibers. Decreased use of the skeletal muscle, induced by the elimination of

the gravitational effects leads to its atrophy (36).
Studies using this methodology have reported that the MND remains unchanged (4, 16) or decreases (4, 19). The study examining the changes induced by inactivity in rats gastrocnemius and tibialis anterior muscles (19) reported in the previous chapter (HS) also observed the effects of 5.4 days of space flight. With this experimental protocol, there was no decrease in the CSA in the TA muscle and the myonuclei number per mm of fiber increased in both muscles; therefore, the values of the cytoplasmic volume per myonucleus decreased (19). The gravitational unloading may have different affects on the muscle structure when compared to the HS-induced atrophy.
In other study (4), with a prolonged experimental protocol (14 days) that examine the myosin heavy chain (MHC) expressed in isolated fibers from the rat soleus muscle, the results support that the cytoplasmic volume per myonucleus decreased in muscle fibers expressing MHC I, and is maintained in fibers co-expressing MHC I and MHC II and those expressing MHC II. The CSA of the fibers expressing MHC I (n=110) and co-expressing MHC I and II (n=44) decreased, but the fibers expressing MHC II (n=27) maintained their CSA. The myonuclei per mm of fiber decreased in the fibers expressing MHC I. These results were obtained using the following methods: a sequence of scans with a confocal microscope was taken trough the entire Z thickness of a fiber section; the myonuclei in the stack for this section were counted and converted into myonuclei per mm by dividing by the length of the field (≈ 173 µm) and multiplying by 1000; the CSA was also calculated from same stack of the section; the mean length of one sarcomere was calculated from 10 consecutive sarcomeres from three different regions; both myonuclei per mm and CSA were adjusted for differences in the sarcomere length (multiplying by the observed sarcomere length and dividing by 2.5 to normalize to a 2.5 µm sarcomere length); the mean cytoplasmic volume per myonucleus was determined by multiplying the fiber CSA by the length of the fiber region (173 µm) and dividing by the number of myonuclei counted in that region (the mean values for each fiber were obtained from three no overlapping regions randomly chosen along the fiber length); fiber ends, damaged regions and markedly stretched (sarcomere lengths > 3.5 µm) were omitted from analysis (4). This study seems to indicate that the fiber types may respond differently to the induced-inactivity.
However, another study using dystrophin antibodies to mark the sarcolema to recognize the underlying myonuclei concluded that the MND in the rat soleus muscle was maintained after 10 days of space flight (16). To recognize the type of skeletal muscle fibers, the authors analysed the myofibrillar ATPase activity. The CSA of both type I and type II fibers (200 muscle fibers) decreased (type I twice as much as type II fibers). The myonuclei were calculated in identified and measured fibers, and its distribution normalized to number per mm of fiber circumference. There were no differences between the control and flight groups in the normalized myonuclei number, indicating that the myonuclei number decreases as the fibers atrophy. The CSA per myonulceus ratio was identical between the control group and the space group. The quantification of fiber CSA and myonuclei was determined from Polaroid pictures (16).
The studies reported in this chapter indicate that differences in the methodological process to determine the main variables to study the MND may induce bias in the results. The latter study (16) determined the myonuclei number normalized to the circumference size of 200 fibers (CSA and myonuclei number were both evaluated in each fiber) (16). This methodological adjustment reported that the type I fibers maintained their MND after the inactivity period, which contrasts with those who reported that fibers expressing MHC I do not maintain it (4).

2.3. Spinal cord transection

Complete transection of the spinal cord induces an atrophic response below the point of lesion in a variety of muscles (36).
In the soleus muscle of adult rats, the effects of inactivity, caused by 4 and 60 days of SCT, have generated a decrease in the MND of the fibers expressing MHC I, MHC IIa, and those expressing both MHC I and MHC IIa (47). The soleus fibers expressing MHC I, MHC IIa and MHC IIx had their MND reduced after 60 days of SCT, but it remained unchanged after four days of SCT, despite the fiber atrophy. The fiber CSA decreased significantly only in the MHC I group after four days of SCT. After 60 days of SCT all the groups of fibers reduced their CSA (MHC I; MHC I+IIa; MHC I+IIa+IIx and MHC IIa). Regarding the myonuclei number per mm of fiber length, only the MHC I group had its myonuclei number reduced after 60 days of SCT. All the other groups maintained the myonuclei number (after four and 60 days of SCT) (47). These results were obtained from isolated fibers and the methodological procedures (4) were already reported.
Another study analysing the MND (cytoplasmic volume per myonucleus) of isolated fibers from the soleus muscle of adult cats demonstrated its decrement after six months of SCT (2). The isolated fibers were typed as slow fibers (expressing MHC I – 100 percent of the fibers in the control group; 86.4 percent in the SCT group) and fast fibers (expressing MHC IIa – 9 percent; and MHC IIb, IIa and I – 4.6 percent in the SCT group). The results showed that the MND of the

control group was significantly larger than the slow fibers of the SCT group. The same conclusion was obtained when the MND of the control group was compared to the MND of the fast fibers in the SCT group. Both fibers' CSA and myonuclei number per mm of fiber decreased during the experimental protocol (2). Once again, these results were obtained with the methodology previously reported (4).

Although previous studies report a tendency in the MND to shrink along the fiber atrophy, it was also documented that some fiber types in the medial gastrocnemius and the tibialis anterior of adult rats maintain their MND (fiber volume per myonuclei number in each field of view). The results were obtained from isolated fibers of each muscle after four and 60 days of SCT (38). The MND quantification was also determined with methods previously reported (4). All fibers were categorized by their MHC expression in type: MHC I; MHC IIa; MHC IIx; MHC IIx + IIb; and MHC IIb. In the medial gastrocnemius, the MND remained unchanged in the group submitted to four days of SCT in the fiber types MHC I; MHC IIa; MHC IIx; and MHC IIx and IIb. The MHC IIb fibers were the only ones whose MND size significantly decreased. The same results were obtained in the group submitted to 60 days of SCT, but the MND also decreased in the fiber type MHC IIx and IIb. In the tibialis anterior, in the group submitted to four days of SCT, all the MND remained unchanged. In the group submitted to 60 days of SCT, only the MND of the fibers type MHC IIb was significantly decreased (there were no IIa fibers in this group, therefore no comparison is possible) (38).

2.5. Denervation

Denervation is another method capable of inducing atrophy (5, 11, 46). Therefore, the use of denervated skeletal muscle is one practicable technique to investigate the change in myonuclei number, fibers' CSA and, consequently, the behaviour of the MND during the atrophy process.

A recent in vivo study (11) directly observed (with a time-lapse imaging technique) the repercussions of 28 days of denervation in rats extensor *digitorius longus* (EDL) and soleus muscles. The results showed that despite the decrement in fibers' CSA, the myonuclei number per fiber was identical throughout the experimental period. These results were obtained by the analyses of fiber segments of 250-1000 µm in different focal planes (5 µm apart each). The levels of apoptosis by TUNEL labelling were also evaluated and the results showed that the inactivity induced high levels of apoptosis; however, when the muscles were stained for laminin and dystrophin, none of the TUNEL-positive nuclei could be categorized as myonuclei (11). These findings showed that the MND seems to diminish after denervation. If the methodological procedure to visualize the skeletal muscle in vivo influences the results (mainly those related to the apoptosis) remain a pertinent question.

In contrast with the previous findings, one study analysing the plantaris muscle of young rats (3 weeks old) reported that 10 days of denervation did not affected the MND (cytoplasmic volume per myonucleus ratio) (46). The muscle CSA decreased after five (519 fibers analysed) and 10 days (417 fibers analysed) of denervation when compared to the control group (432 fibers analysed). The same results were obtained when the myonucleus number per mm was evaluated. These findings were obtained from isolated fibers. The fiber CSA and cytoplasmic volume were determined in images by the use of formulas (CSA = 3.14 x (w/2)2; cytoplasmic volume =CSA x l; w and l are the width and length of the fibers measured on the image, respectively) (46).

The MND in the diaphragm muscle is also a referred matter in various studies (5, 28). In type-identified single diaphragm fibers (classified as type I, IIa, IIx or IIb on the basis of MHC isoform), the MND (fiber volume per myonucleus) increases in the type I fibers, is maintained in the type IIa fibers and decreases in the type IIx and IIb fibers during two weeks of denervation (5). The fibers' CSA and volume were assessed and averaged from optical sections (three different positions along an arbitrarily selected 300 µm length of the fiber). The myonuclei number and the sarcomeric spacing were calculated in each fiber section. The total myonuclei number per fiber was calculated from the mean myonuclei number per micrometer (after adjustment to a 2.5 µm sarcomeric length) and normalized to 2 cm of fiber (5). These results contrast with those reporting that the MND remains stable after 12 hours of mechanical ventilation (28). Although the mechanism to induce atrophy and the time of the experimental protocol are different, the myonuclei apoptosis is present even in just a short period of time. This apoptotic behaviour of the diaphragm muscle myonuclei contrasts with the *in vivo* study findings that reported the absence of apoptotic myonucleus in the EDL and soleus muscle (11).

2.6. Bed rest

One study analysing the effects of two and four months of bed rest in the soleus muscle of humans (31) (six subjects) reported that the MND (cytoplasmic volume per myonucleus) decreases only in the fourth month of bed rest. The fibers' CSA only decreases in the fourth month of bed rest and the myonuclei number per mm are maintained throughout the four months (31). All the methods utilized to evaluate the main variables to examine the MND were previously

reported (4).

In conclusion, it appears that during periods of inactivity the skeletal muscle may respond through the following processes: 1) the reduction in the fibers' CSA, maintenance or reduction of the myonuclei number and, therefore, smaller MND producing an higher than normal complement of DNA; 2) the adaptations in the fibers' CSA seems to occur faster than those in the myonuclei number, i.e., the myonuclei number per fiber seems to decrease at a lower rate than the CSA, indicating that the adaptation of the skeletal muscle fibers to inactivity is modulated not only by changes in the amount of gene expression in the existing myonuclei, but also by alterations in the total myonuclei number available; 3) the modulation in the quantity of gene expression and protein production among the myonuclei seems to indicate an early and more adaptive strategy (faster reduction in the CSA); and 4) the modulation in the myonuclei number seems to be a second and more chronic adaptive strategy.

3. THE MND DURING THE MUSCLE HYPERTROPHY PROCESS

The MND during the skeletal muscle hypertrophy has been addressed in several studies (6, 13, 18, 25, 27, 34, 35, 37, 45). The underlying question during this state of skeletal muscle adaptation is to determine if the enlargement of the cytoplasm is accompanied with the incorporation of new myonuclei – therefore the total quantity of genetic apparatus available for protein synthesis would be amplified with small alterations in the dynamics of protein production of each myonucleus; maintaining the MND – or if the existing myonuclei are able to maintain the expression and distribution of the increased request of proteins. Each myonucleus could basically increase its protein production for the greater volume of cytoplasm, therefore enlarging the MND (3, 8). Interestingly, a recent debate about this issue has gathered the opinion of various authors but a final conclusion has not been achieved (7, 9, 15, 17, 24, 26, 29, 33). Various factors can determine if the addition of satellite cells is obligatory for the skeletal muscle hypertrophy. These factors include type of growth stimulus, extent of growth response, age, specie and growth phase of the animal, and time of samples collection after the applied stimulus (30).

3.1. Sinergist ablation

This skeletal muscle hypertrophying mechanism induces an increased loading in a determined muscle by the surgical ablation of its synergist muscles. In response, the CSA and mass of the selected muscle increases (36).

The alterations induced by this mechanism over three weeks were studied in the rat soleus muscle fibers (27). The results showed that the mean muscle CSA of the functional overloaded group (FO) was not significantly different from the control group, but the weight of the muscle was increased. The mean myonuclei number increased and the mean MND (cytoplasmic volume per myoncleus) was identical to that in the control group. The results were obtained from mechanically isolated (microdissected) fibers. Along the proximal, middle and distal portions of the fiber, three regions (173 µm) were observed and averaged to determine the CSA, myonuclei number per mm and cytoplasmic volume per myonucleus of the fiber. One hundred seventy-eight and 186 fibers were analysed from the control and FO groups respectively (27). All the other processes to determine the MND were described previously (4). This study concluded that, during FO, the increase in the myonculear number and fiber size were combined so the MND was preserved. The same conclusion was obtained for the EDL muscle of rats. Four weeks of FO caused hypertrophy, characterized by a significant augment in muscle mass and a proportional increase in the myonuclei number (35).

The plantaris muscles (isolated fibers) of adult rats were also evaluated after 10 weeks of FO (37). The results showed that the fibers' CSA of FO group (19 fibers analysed) increased compared to the control group (13 fibers analysed). The same outcome was observed in the myonuclei number per mm of fiber. The MND (cytoplasmic volume per myonucleus) was maintained in the type I and type IIa fibers but decreased in the type IIx/b fibers (37). The methods used for the quantification of the variables were previously described (4). The same effect was produced in the plantaris muscle of cats after the application of three months of FO (2). The results showed an increase in the slow (22 fibers analysed) and fast (53 fibers analysed) fibers' CSA compared to each control group (nine and 37 fibers analysed of the slow and fast control groups, respectively). The myonuclei number per mm of fiber also increased in both groups. The MND (cytoplasmic volume per myonucleus) was maintained in the slow fiber types but decreased in the fast ones (2).

Again, the analyzed data suggests the presence of regulatory mechanisms that guarantee a thigh coupling between the amount of the fiber genetic apparatus and protein requirements.

3.2. Strength training

Although the MND was not calculated, one study analysing the trapezius of 10 elite power lifters (PL) demonstrated a significant correlation between the myonuclear number and the fibers' CSA (18). The muscle biopsies also revealed that the fibers' CSA and

the myonuclei number per fiber were significantly higher in PL compared to the control group. All the data was collected using a light microscope connected to a computerised image analysis system. Nearly 200 fibers were photographed and utilized for the quantification of fiber CSA and myonuclei number (18). These findings suggest that larger fibers would contain more myonuclei, reinforcing the MND concept.

4. MND ACCORDING TO THE FIBER TYPE COMPOSITION, AGE AND BODY SIZE

4.1. Fiber type composition

The examination of the relationship between the fibers' CSA and its myonuclei number during the skeletal muscle atrophy (2, 4, 5, 16, 38, 47) and hypertrophy (2, 37) indicate that the changes in these two variables differ according to the muscle fiber type composition.

Six months of atrophy (induced by SCT) in the soleus muscle of cats changed the fibers' MHC expression (2). All the control fibers expressed MHC I, but after the experimental protocol the expression changes toward a faster profile (84.6 percent of the SCT group expressed MHC I, 9 percent expressed MHC IIa and 4.6 percent MHC IIb + IIa + I). Since there were no fast fibers in the control group, the authors compared the fast fibers with the slow fibers in the SCT group. The results showed that the fast fibers had less myonuclei number per mm of fiber but there were no differences in the fibers' CSA and in the MND (cytoplasmic volume per myonucleus). It seems that the prolonged period of atrophy may induce changes in the MHC expression.

Another study analysing the soleus muscle in rats using 10 days of space flight to induce atrophy reported a greater myonuclei number in the slow (type I) fibers compared with the fast (type II) fibers (16). The effects on the fibers' CSA was also fiber type-dependent; therefore, the results showed that atrophy has a bigger effect on slow than fast fibers. Supporting these results, a study with the same experimental design (14 days of space flight), also reported that the extent of atrophy (fibers' CSA) was greater in the slow fibers (expressing MHC I and co-expressing MHC I and II) than on the fast fibers (expressing MHC II) (4). Interestingly, the myonucleus number per mm was significantly lower in the fibers expressing MHC I of flight animals (those that had a greater reduction in the fiber CSA), but was maintained in the fibers co-expressing MHC I and II (a soft decrease in their fiber CSA was observed compared to the fibers expressing MHC I), and in the fibers expressing MHC II (4). These results indicate a close relationship between the myonucleus number and the CSA in both slow and fast fibers. Moreover, this study also reported that during the atrophy process the fibers MHC expression is altered toward a faster fiber phenotype.

Another study analysing the repercussions of four and 60 days (47) (see the atrophy chapter) of SCT in the soleus muscle of rats showed that the fibers expressing MHC I possibly were more affected by inactivity than those fibers co-expressing MHC I and IIa, MHC I and IIa and IIx, and MHC IIa. Regarding the myonuclei number per mm of fiber length, the fibers expressing MHC I were the only group with a significant reduction after 60 days of SCT. The results of the MND (fiber volume per myonucleus) indicate that the fibers expressing the faster types of MHC (IIa, IIx, IIb) are those who have the greater MND. In this study there was also a tendency in the muscles fibers to change their MHC expression into a faster type (47). Similar results, with the same time of SCT, were also reported in the Gast and TA muscles of rats (38).

Interestingly, although maintaining the similar characteristics of the other muscle fibers (fiber CSA, myonuclei number and MND), the faster fibers of the diaphragm are apparently more responsive to the atrophy process that the slow ones (5). This may indicate that possible gravitational effects influence the muscle reaction to the induced inactivity.

In summary, during the atrophy process the fibers MHC expression is altered toward a faster fiber phenotype (4, 47). Given that fibers expressing faster MHC phenotypes appear to have greater CSA and fewer myonuclei per mm, this time-dependent response may bias the MND results. Studies analysing the MND during the hypertrophy process also reported that the slow fibers had a smaller MND than the fast ones (2, 37). One study verified this outcome after the induction of a FO+ (synergist ablation plus exercise) stimulus in the rat plantaris muscle. The results indicated that the cytoplasmic enlargement of the skeletal muscle fibers was followed by an increase in the myonuclei number. Nevertheless, in the FO group without exercise, all the fiber types had a similar MND. This effect seems to be related to a relatively greater increase in the myonuclei number compared to the fibers CSA (37). Similar results were reported in a study analysing the effects of FO in the plantaris muscle of cats. The MND was also similar between the FO slow and fast fibers (2).

Summarizing, all the reported results seem to point out the possible role of the fiber metabolic status in determining its CSA, myonuclei number and MND, which results in the tendency of the fast (probably low oxidative) fibers to have the largest MND compared to slow (high oxidative) fibers. The main metabolic pathway utilized by the fiber seems to be a significant determinant of its morphology and myonuclear

number. Studies demonstrated that the fibers' CSA and myonuclei number may be correlated with the activity of central enzymes in the metabolic pathways (36, 43). These differences in the myonuclear density, fibers' CSA and MND may reveal in part the different demands in the metabolic and protein expression arrangements in the diverse fiber types. The smallest MND in slow fibers may also be related to the quicker contractile and metabolic protein turnover rates in mainly slow muscles compared to fast ones (22, 43).

4.2. Age and body size

The relationship between the fibers' myonuclei number and the CSA throughout the age of the subjects was also addressed in some studies (21, 45).

The analyses of the TA muscle (127 fibers) of newborn mice and mice at 1 (355 fibers), 3 (305 fibers), and 5 (428 fibers) weeks, and 6 (177 fibers) and 18 (378 fibers) months old, demonstrated that the MND (fiber volume per myonucleus) increases during the growth phase (birth to 6 months old) and decreases during the aging phase (6 to 18 months old) (45). In the period between 5 weeks and 6 months of age, the results showed that the fibers' hypertrophy is followed by a decrease in the myonuclei number. In the oldest mice (18 months), no correlation was found between the myonuclei number and fiber CSA, but throughout the remaining ages this correlation was found, especially in the mice at 1 week old (r=0.70). This outcome may be related to the fact that, despite a reduction in the fibers' CSA of the 18-months-old group, the myonuclei number was similar between them and the 6-months-old group (45). All the methodological processes were previously reported (46).

Another study analysing the MND (fiber CSA per myonucleus) in the soleus muscle of rats with different ages (6 and 32 months) reported that the fibers' CSA were significantly smaller in the oldest rats but the myonuclei number per fiber was similar between the two groups (21). Consequently, the MND was smaller in the oldest group than in the youngest. The effects of HS (14 days) were also analysed. The results showed that, despite a reduction in the CSA, the oldest rats maintained their myonuclei number per fiber, and consequently, their MND was smaller (21).

Both studies indicate that the aging process is associated with maintenance of the myonuclei number per fiber, possibly because the responsible mechanisms for their elimination are less reactive. This supposition is based in the observation of high levels of apoptosis in the soleus muscle of old rats without the elimination of their myonucleus (this elimination happened in the young rats) (21).

To clarify whether the MND is related to the size and mass of the animal, experiments were done in different species with different body masses (from 25g to 2500Kg) (23). A total of 174 single fibers (two points per fiber from 3D reconstructions and at a set sarcomere length) were analysed. The results showed that the MND (fiber volume per myonuclei number) is positively correlated with the body mass of the subject and it is very dependent on muscle fiber type (the fast fibers had the largest MND) (23).

5. CONCLUSIONS

Some conclusions can be drawn from this review: 1) changes in the skeletal muscle size appear to be related to the MND (fibers volume and myonuclei number); 2) the MND is fiber type-specific; 3) during muscle atrophy, both myonuclei number and MND decrease seems to be important to produce muscle shrinkage; 4) during hypertrophy, the main system for the enlargement of the fibers seems to be the increase in the myonuclei number (the MND in normal conditions seems to be functioning near a "ceiling" of the transcriptional levels, i.e., each myonucleus seems to be functioning near its maximum transcriptional potential; 5) the different experimental procedures utilized to quantify the fibers' CSA and, mainly myonuclei number, may be inducers of errors; 6) when studying the MND the following issues may determine the final results: age, race and size of the subjects; kind of stimulus inducted to produce muscle changes; sampling time (muscles undergoing changes may induce errors); and time of the study (application of the stimulus).

The fact that the MND is one theoretical concept may be responsible for the controversy around this issue. In fact, the methodological procedure used to calculate this variable is based on the relationship between the fibers' myonuclei number/density and their CSA/volume. Nevertheless, the concept of MND is based on the assumption that each myonucleus in the muscle fiber may be responsible for the regulation of protein synthesis for its surrounding area. Consequently, this controversy may be solved theoretically with studies analysing each myonuclei protein production (staining specific proteins from their myonucleus of origin).

This review may provide important insight into how muscle fibers adjust to meet the demands of protein synthesis or degradation in response to different metabolic, physiological and biochemical requirements. Specifics of the structural and functional adjustments that occur during atrophy and hypertrophy processes as well as mechanistic explications for how these changes occur are lacking. Nevertheless, several studies addressing the MND issue appear to establish an important role for modulation of myonuclear number in diverse skeletal muscle adaptation processes. Therefore, changes in myonuclear number

or MND size are linked to and appear to be essential for changes in myofiber size.

During skeletal muscle hypertrophy, it seems that the prevailing mechanism for increasing fiber size is the increase in myonuclear number. This proposal suggests that the MND size under normal conditions may be operating at or very close to the highest overall transcriptional levels, or instead, that diffusion distances are restraining, requiring the addition of more DNA units to increase successful distribution of gene products during fiber hypertrophy. Likewise, during skeletal muscle atrophy, the increased rate of proteolysis and/or decreased protein synthesis seems to be balanced by the rate of myonuclei loss by apoptosis, necrosis or aponecrosis. Therefore, it appears that the decreased transcriptional demands lead to the decrease in the myonuclei number. These findings suggest that the response of skeletal muscle tissue to diverse external stimuli is always seeking a balance between the DNA available for growing or shrinking situations.

6. REFERENCES

1. Allen DL, Linderman JK, Roy RR, Grindeland RE, Mukku V, Edgerton VR. Growth hormone IGF-I and/or resistive exercise maintains myonuclear number in hindlimb unweighted muscles. *J Appl Physiol* 1997; 83: 1857-1861.
2. Allen DL, Monke SR, Talmadge RJ, Roy RR, Edgerton VR. Plasticity of Myonuclear Number in Hypertrophied and Atrophied Mammalian Skeletal-Muscle Fibers. *J Appl Physiol 1995;*78: 1969-1976.
3. Allen DL, Roy RR, Edgerton VR. Myonuclear domains in muscle adaptation and disease. *Muscle Nerve 1999;* 22: 1350-1360.
4. Allen DL, Yasui W, Tanaka T, Ohira Y, Nagaoka S, Sekiguchi C, Hinds WE, Roy RR, Edgerton VR. Myonuclear number and myosin heavy chain expression in rat soleus single muscle fibers after spaceflight. *J Appl Physiol 1996;* 81: 145-151.
5. Aravamudan B, Mantilla CB, Zhan W-Z, Sieck GC. Denervation effects on myonuclear domain size of rat diaphragm fibers. *J Appl Physiol* 2006; 100: 1617-1622.
6. Barton-Davis ER, Shoturma DI, Sweeney HL. Contribution of satellite cells to IGF-I induced hypertrophy of skeletal muscle. *Acta Physiol Scand* 1999; 167: 301-305.
7. Bodine SC. Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol 2007;* 103: 1105-1106.
8. Boonyarom O and Inui K. Atrophy and hypertrophy of skeletal muscles: structural and functional aspects. *Acta Physiol* 2006; 188: 77-89.
9. Booth FW. Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol* 2007; 103: 1105-1105.
10. Brack AS, Bildsoe H, Hughes SM. Evidence that satellite cell decrement contributes to preferential decline in nuclear number from large fibres during murine age-related muscle atrophy. *J Cell Sci* 2005; 118: 4813-4821.
11. Bruusgaard JC, Gundersen K. In vivo time-lapse microscopy reveals no loss of murine myonuclei during weeks of muscle atrophy. *J Clin Invest* 2008; 118: 1450-1457.
12. Dupont-Versteegden EE. Apoptosis in muscle atrophy: relevance to sarcopenia. *Exp Gerontol* 2005; 40: 473-481.
13. Dupont-Versteegden EE, Murphy RJ, Houle JD, Gurley CM, Peterson CA. Mechanisms leading to restoration of muscle size with exercise and transplantation after spinal cord injury. *Am J Physiol Cell Physiol* 2000; 279: C1677-1684.
14. Hall ZW, Ralston E. Nuclear Domains in Muscle Cells. *Cell* 1989; 59: 771-772.
15. Hikida RS. Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol* 2007; 103: 1104-1105.
16. Hikida RS, VanNostran S, Murray JD, Staron RS, Gordon SE, Kraemer WJ. Myonuclear loss in atrophied soleus muscle fibers. *Anat Rec* 1997; 247: 350-354.
17. Kadi F. Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol* 2007; 103: 1105-1105.
18. Kadi F, Eriksson A, Holmner S, Butler-Browne GS, Thornell LE. Cellular adaptation of the trapezius muscle in strength-trained athletes. *Histochem Cell Biol* 1999; 111: 189-195.
19. Kasper CE and Xun L. Cytoplasm-to-myonucleus ratios following microgravity. *J Muscle Res Cell Motil* 1996; 17: 595-602.
20. Kasper CE, Xun L. Cytoplasm-to-myonucleus ratios in plantaris and soleus muscle fibres following hindlimb suspension. *J Muscle Res Cell Motil* 1996; 17: 603-610.
21. Leeuwenburgh C, Gurley CM, Strotman BA, Dupont-Versteegden EE. Age-related differences in apoptosis with disuse atrophy in soleus muscle. *Am J Physiol Regul Integr Comp Physiol* 2005; 288: R1288-1296.
22. Lewis SEM, Kelly FJ, Goldspink DF. Pre-Natal Post-Natal Growth and Protein-Turnover in Smooth-Muscle, Heart and Slow-Twitch and Fast-Twitch Skeletal-Muscles of the Rat. *Biochem J* 1984; 217: 517-526.
23. Liu JX, Hoglund AS, Karlsson P, Lindblad J, Qaisar R, Aare S, Bengtsson E, Larsson L. Myonuclear domain size and myosin isoform expression in muscle fibres from mammals representing a 100,000-fold difference in body size. *Exp Physiol* 2009; 94: 117-129.
24. Lowe DA. Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol* 2007: 103: 1106-1106.
25. Lowe DA, Alway SE. Stretch-induced myogenin, MyoD, and MRF4 expression and acute hypertrophy in quail slow-tonic muscle are not dependent upon satellite cell proliferation. *Cell Tissue Res* 1999; 296: 531-539.
26. Mantilla CB and Sieck GC. Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol* 2007; 103: 1104-1104.
27. McCall GE, Allen DL, Linderman JK, Grindeland RE, Roy RR, Mukku VR, Edgerton VR. Maintenance of myonuclear domain size in rat soleus after overload and growth hormone/IGF-I treatment. *J Appl Physiol* 1998; 84: 1407-1412.
28. McClung JM, Kavazis AN, DeRuisseau KC, Falk DJ, Deering MA, Lee Y, Sugiura T, Powers SK. Caspase-3 Regulation of Diaphragm Myonuclear Domain during Mechanical Ventilation-induced Atrophy. *Am J Respir Crit Care Med* 2007; 175: 150-159.
29. O'Connor RS, Pavlath GK. Point: Counterpoint: Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol* 2007; 103: 1099-1100.
30. O'Connor RS, Pavlath GK, McCarthy JJ, Esser KA. Last Word on Point: Counterpoint: Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol* 2007; 103: 1107-1107.
31. Ohira Y, Yoshinaga T, Ohara M, Nonaka I, Yoshioka T, Yamashita-Goto K, Shenkman BS, Kozlovskaya IB, Roy RR, Edgerton VR. Myonuclear domain and myosin phenotype in human soleus after bed rest with or without loading. *J Appl Physiol* 1999; 87: 1776-1785.
32. Pavlath GK, Rich K, Webster SG, Blau HM. Localization of muscle gene products in nuclear domains. *Nature* 1989; 337: 570-573.
33. Rehfeldt C. Satellite cell addition is/is not obligatory for skeletal muscle hypertrophy. *J Appl Physiol* 2007; 103: 1104-1104.

34. Rosenblatt JD, Parry DJ. Gamma irradiation prevents compensatory hypertrophy of overloaded mouse extensor digitorum longus muscle. *J Appl Physiol* 1992; 73: 2538-2543.
35. Rosenblatt JD, Yong D, Parry DJ. Satellite Cell Activity Is Required for Hypertrophy of Overloaded Adult Rat Muscle. *Muscle Nerve* 1994; 17: 608-613.
36. Roy RR, Baldwin KM, Edgerton VR. The plasticity of skeletal muscle: effects of neuromuscular activity. *Exerc Sport Sci Rev* 1991; 19: 269-312.
37. 37. Roy RR, Monke SR, Allen DL, Edgerton VR. Modulation of myonuclear number in functionally overloaded and exercised rat plantaris fibers. *J Appl Physiol* 1999; 87: 634-642.
38. Roy RR, Zhong H, Siengthai B, Edgerton VR. Activity-dependent influences are greater for fibers in rat medial gastrocnemius than tibialis anterior muscle. *Muscle Nerve* 2005; 32: 473-482.
39. Schmalbruch H, Lewis DM. Dynamics of nuclei of muscle fibers and connective tissue cells in normal and denervated rat muscles. *Muscle Nerve* 2000; 23: 617-626.
40. Smith HK, Maxwell L, Martyn JA, Bass JJ. Nuclear DNA fragmentation and morphological alterations in adult rabbit skeletal muscle after short-term immobilization. *Cell Tissue Res* 2000; 302: 235-241.
41. Suzuki I, Komiya H, Maeda J, Kasuga N, Takemiya T. Fiber Type Specificity, Size, Myonuclear Number and Domain in Slow and Fast Muscles in Adult Mice. *Adv Exerc Sports Physiol* 2006; 12: 35-436.
42. Tarakina MV, Turtikova OV, Nemirovskaia TL, Kokontsev AA, and Shenkman BS. The contribution of muscle progenitor cells to maintaining morphological characteristics of unweighted rat soleus muscle during passive stretch. *Tsitologiia* 2008; 50: 140-146.
43. Tseng BS, Kasper CE, Edgerton VR. Cytoplasm-to-myonucleus ratios and succinate dehydrogenase activities in adult rat slow and fast muscle fibers. *Cell Tissue Res* 1994; 275: 39-49.
44. Verheul AJ, Mantilla CB, Zhan WZ, Bernal M, Dekhuijzen PNR, Sieck GC. Influence of corticosteroids on myonuclear domain size in the rat diaphragm muscle. *J Appl Physiol* 2004; 97: 1715-1722.
45. Wada KI, Katsuta S, Soya H. Natural occurrence of myofiber cytoplasmic enlargement accompanied by decrease in myonuclear number. *Jpn J Physiol* 2003; 53: 145-150.
46. Wada KI, Takahashi H, Katsuta S, Soya H. No decrease in myonuclear number after long-term denervation in mature mice. *Am J Physiol Cell Physiol* 2002; 283: C484-488.
47. Zhong H, Roy RR, Siengthai B, Edgerton VR. Effects of inactivity on fiber size and myonuclear number in rat soleus muscle. *J Appl Physiol* 2005; 99: 1494-1499.