## Mitochondrial Size Distribution Analysis in the Soleus Muscle of Trained and Aged Rats

The capacity of muscles to oxidize pyruvate, activities of enzymes of the mitochondrial electron transport chain, concentration of cytochrome c, and mitochondria protein are seen to increase in rat muscles subjected to a strenous exercise program [1]. A shift in the size distribution to smaller mitochondria has been demonstrated in rat myocardium with nearly identical training techniques [2]. This shift in the direction of smaller mitochondria has been hypothesized to increase the surface to volume ratio in response to maturation and training.

It was the purpose of this study to investigate aging and training induced alterations in mitochondrial size distribution of soleus muscle without regard to their specific subcellular localization.

Twelve male rats from the Charles River Breeding Laboratories were taught to run on a motor-driven treadmill for 3 days. At the termination of the initial learning period, the animals were randomly divided into sedentary and running groups. The experimental group was trained to run to 2,100 m/day at 30 m/min on a 10% incline. The total training period was continued for 16 weeks and all animals were fed standard laboratory chow and were given water ad libitum.

The animals (including an immature and 720-day-old animal) were not exercised for 24 h prior to sacrifice at which time to soleus was rapidly removed. A section from the belly of the soleus was taken for electron microscopic examination as previously described [3].

Electron micrographs taken at a magnification of $\times 6{,}000$ were examined by projecting them on to a screen and size typing individual mitochondria with a particle size analyzer. The mitochondria were partitioned into 9 categories corresponding to absolute sizes of $1.7 \times 10^{-2}$, $3.8 \times 10^{-2}$, $6.8 \times 10^{-2}$, $15.3 \times 10^{-2}$, $27.2 \times 10^{-2}$, $42.4 \times 10^{-2}$, $61.1 \times 10^{-2}$, $83.2 \times 10^{-2}$, and $108.6 \times 10^{-2}$ $\mu m^2$.

The results indicate that the greater percentage of mitochondrial numbers and percentage area are of size types 6 and 7. These size types comprise 56.0 to 66.1% of the total percent numbers and 48.5 to 60.3% of the total percent area. The larger mitochondrial size types 1 and 2 account for 0.7 to 1.7% of the numbers and comprise 3.1 to 7.4% of the percentage area. Conversely, the smallest mitochondria (sizes 8 and 9) comprise from 17.1 to 25.3% of the percent number but only 4.8 to 8.2 percent of the area.

Comparing trained and non-trained, it can be seen that the trained animals exhibit a higher percentage of size 6 and 7 mitochondria in both percent number and percent area and less percentages of 8 and 9 size types. The percent number and percent area of the larger mitochondria remained nearly the same. The soleus from the

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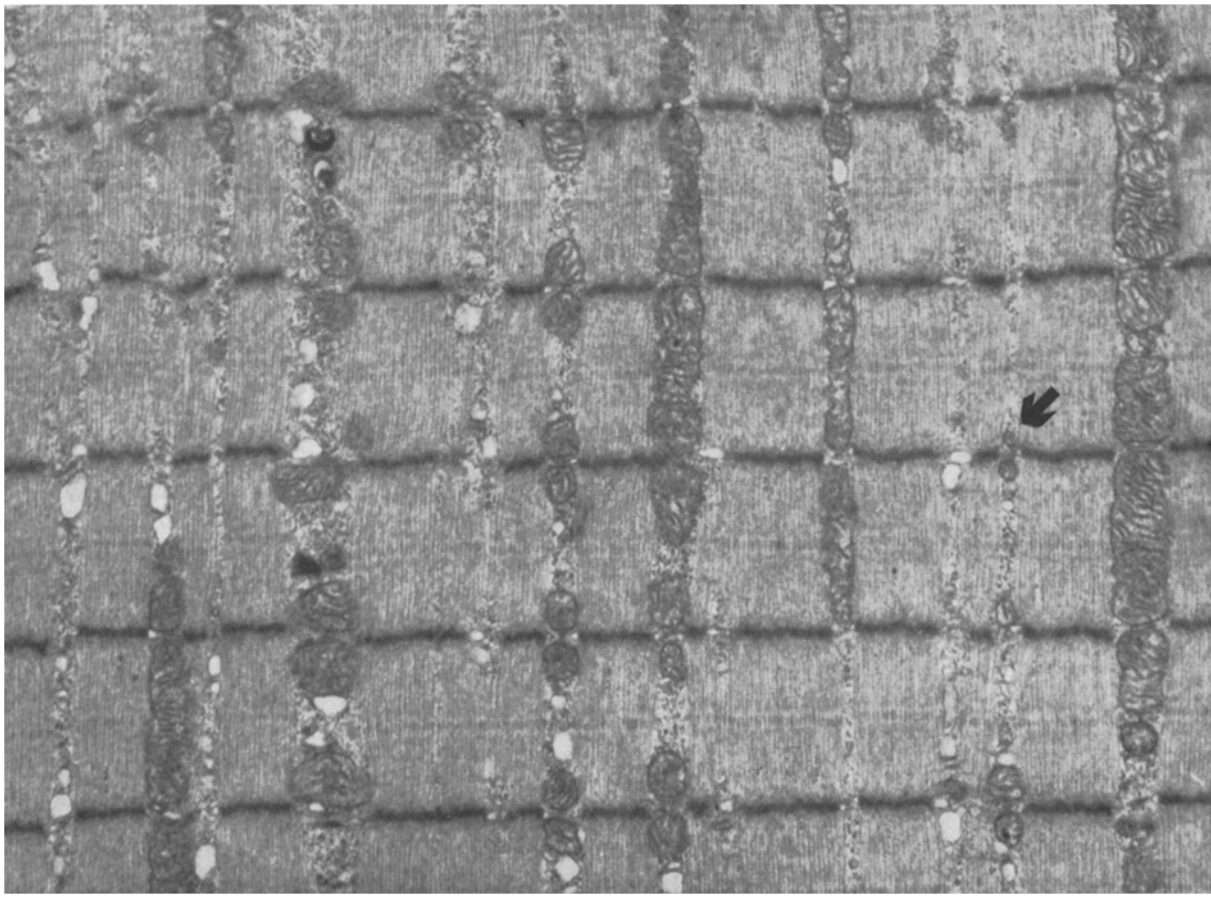

Illustration of pre-functional, precursor mitochondria in the soleus muscle; $\times 20{,}000$.

trained animal represents more closely that distribution from the 50 day, immature rat in relation to the low number of 8 and 9 and high percentages of size types 6 and 7. This would be in agreement with the recent heart studies in our laboratory where a training induced shift to the size types corresponding to 6 and 7[3].

In the heart the smallest size mitochondria are of size 7 and maturation and training resulted in a shift towards this size. The present data clearly show that for all ages the predominate size mitochondria in the soleus muscle is of size types 6 and 7. Due to the non-existance of mitochondrial size types 8 and 9 in the heart and the results of our previous studies we have hypothesized that: 1. there exists an 'optimal' mitochondrial size type in the soleus and 2. the mitochondria of size types 8 and 9 are precursor mitochondria.

Percent number and percent area distributions of mitochondria by size grouping

| Group (N) | Size categories[a] | | | |
|---|---|---|---|---|
| | 1,2 | 3,4,5 | 6,7 | 8,9 |
| | Percent of total mitochondrial number | | | |
| 50 (2) | 1.1 | 17.0 | 66.1 | 15.9 |
| Mature non-trained (6) | 1.7 | 18.6 | 56.0 | 23.7 |
| Mature trained (6) | 1.7 | 19.8 | 61.4 | 17.1 |
| 720 (1) | 0.7 | 13.6 | 60.4 | 25.3 |
| | Percent of total mitochondrial area | | | |
| 50 (2) | 4.8 | 34.9 | 56.1 | 4.2 |
| Mature non-trained (6) | 7.4 | 38.0 | 48.5 | 6.2 |
| Mature trained (6) | 5.9 | 37.7 | 51.5 | 4.8 |
| 720 (1) | 3.1 | 28.4 | 60.3 | 8.2 |

[a] Size categories correspond to absolute sizes ranging from $1.7 \times 10^{-2}$ to $108.6 \times 10^{-2}$ $\mu m^2$.

The older soleus muscle samples (720 day) tend to have a high percent number and area of size type 8 and 9 mitochondria, similar to the mature non-trained animals. If the aging muscle cell is in a state of non-growth, low energy requirement, this could account for the increase in these size types with age.

It appears that the training either resulted in the formation of less smaller mitochondria, or that the smaller mitochondria are precursor mitochondria to the predominate size types in the muscle cell. This could account for the higher percent mitochondria in the medium and larger size types, as less smaller mitochondria are found in trained muscle. It is a possibility that these smaller mitochondria are non-functional and are only precursors to the larger size mitochondria within the muscle cell.

We can hypothesize that in the soleus muscle cell an 'optimal' mitochondrion size is existent, and there exists pre-functional mitochondrial. These optimal mitochondrial sizes could be influenced by both cell function and by subcellular localization. Further analysis of soleus mitochondrial data with regard to specific subcellular localization is now in progress.

*Résumé.* Analyse par microscopie électronique du muscle jambier postérieur chez des rats âgés de 50 jours, adultes, avec ou sans entraînement physique et âgés de 720 jours. Les résultats laissent supposer l'existence d'une taille optimum pour les mitochondries de même que la présence de mitochondries avant-courrières.

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## A Comparative Study of the Correlations Existing in the Ratio Between Nucleolar Dimensions and Number of Nucleolini in the Oocytes of Amphibia

*Introduction and methods.* As early as 1910, Cajal[1], had been able to show that the nucleolus contained argentophil spherules, which he called 'intranucleolar spherules'. In addition to these structures, the author had been able to ascertain the presence of nucleolar vacuoles, isolated granules and the nucleolini noted by many authors[2]. The nucleolini, which are found in relation to particular functional stages of the nucleolus[3,4], can be made to demonstrate well with the method of platinum chloride impregnation[5]. They are also present in the cells in vivo and have the characteristic of being highly refractive[3,6,7].

In the oocytes of *Bufo vulgaris*, Albanese and Bolognari[8] had been able to ascertain, by statistical examination, that there is a correspondence between the nucleolar granules and the diameter of the nucleoli.

Studies carried out also along these lines by Bolognari and Caminiti[9] on mononucleolate oocytes of *Echinus melo* confirmed that these nucleolini, which are absent in the smallest nucleoli, become increasingly numerous as the volume of the nucleoli increases. In order to see whether this behaviour is to be found in other species, we turned our attention to the oocytes of 2 Anouran Amphibians, *Rana esculenta* and *Discoglossus pictus*, and those of a Urodelan Amphibian, *Triturus cristatus*. We carried out a statistical examination, adopting the same criteria that were used previously[8], so that we could also make a comparison between the 3 species of Amphibians. For the statistical examination, carried out on preparations treated by the method of impregnation with platinum chloride, 400 nucleoli were considered for each species, the number of nucleolini present in each nucleolus being counted and their maximum diameter measured.

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