A MORPHOMETRIC STUDY ON HUMAN MUSCLE MITOCHONDRIA IN AGING

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ABSTRACT

Mitochondria are dynamic organelles capable of significant changes of their ultrastructural features according to the tissue-specific energy demands. In human biopsies of vastus lateralis and anterior tibialis muscles from young (25.0 ± 4.4 years), middle-aged (50.4 ± 7.5 years) and old (75.5 ± 3.9 years) healthy volunteers, we carried out a morphometric study on subsarcolemmal and intermyofibrillar mitochondria to assess whether age-related alterations of the morphology of these organelles contribute to the muscle performance decay in aging. By computer-assisted methods, we measured: the average area (MAA), the longer diameter (Dmax) and the ratio perimeter to area (pleomorphic index: Plei) of mitochondria. No significant age-related ultrastructural differences were found either in subsarcolemmal or intermyofibrillar organelles. However, in middle-aged as well as in the old group of patients vs. the young one, MAA and Dmax showed a clear trend to decrease, while Plei showed a marked, age-related tendency to increase. Higher percentages of less pleomorphic organelles were found in the youngest group of patients and this was particularly evident in the subsarcolemmal mitochondrial population. In addition to reporting on discrete aspects of mitochondrial ultrastructure, MAA, Dmax and Plei are closely related to each other and provide a reliable index of the muscle mitochondria adaptive response to age. Thus, we interpret our results as indicating a substantial preservation of muscle mitochondrial ultrastructure during aging.

INTRODUCTION

In elderly people, physiological muscle atrophy constitutes a common, macroscopic alteration (1). Investigations on carefully selected, old human beings documented significant correlations between reduction in muscle strength (both isometric and dynamic) and decrease in fibre number and size, specifically the fast twitch ones: type II (2). These functional features are consistent with a significant age-related decay in the activity of several mitochondrial enzymes reported both in muscles from human beings (3) as well as laboratory animals (4). With reference to human studies, also the number of mitochondria per gram of tissue decreases in subjects over 50 years of age (5, 6). The functional outcome of these changes is a marked reduction in aerobic endurance which appears to be correlated to mitochondrial density and to the specific metabolic competence of each organelle in providing adequate amounts of adenosinetriphosphate (ATP) (7).

As documented by electron microscopic studies in different tissues, the ultrastructural features of a given mitochondrial population are coupled to the organelles'

functional performances and can undergo significant adaptations to the changing environmental conditions (8-11). Thus, quantitation of mitochondrial morphological parameters, e.g. average area, longer diameter and the ratio perimeter to area (pleomorphic index) of the organelles, while enabling the estimation of the cell potential for energy provision, can measure eventual changes underlying an impaired metabolism. To seek age-related signs of an altered energy metabolism, we carried out a computer-assisted morphometric study on muscle mitochondria in human biopsies from young, middle-aged and old healthy volunteers. Intermyofibrillar and subsarcolemmal organelles were analysed separately in muscle pieces conventionally processed for electron microscopy (Fig. 1).

RESULTS

Fig 2 reports the results of subsarcolemmal mitochondria: although no significant difference was found among the age groups taken into account, with advancing age, MAA and Dmax showed a clear trend to decrease and Plei to increase, respectively. The data on intermyofibrillar mitochondria, reported in Fig 3, document the same not significant changes and trend. The percent distributions of Plei values from both the mitochondrial populations (Figures: 4, 5) report that young patients are characterised by higher percentages of less pleomorphic organelles: a feature which is particularly evident in the subsarcolemmal population (Fig. 4).

DISCUSSION

Although we did not find significant age-related differences in both the muscle mitochondrial populations investigated, a decreasing trend was apparent for MAA and Dmax in middle-aged and old patients. In these groups, higher percentages of more convoluted and distorted organelles (Plei histograms and distributions in Figs. 2-5) were also evident. The present findings confirm previous reports documenting a substantial lifespan preservation of muscle mitochondria ultrastructural features not only in physiological aging, but also in a severe pathological condition, such as chronic fatigue syndrome, where a decline of the mitochondrial metabolic competence is reported to be a typical alteration (12, 13).

Some considerations must be made with reference both to the specific location of the sampled organelles and the functional significance of the parameters taken into account. Subsarcolemmal mitochondria provide energy for active transport processes across the muscle membrane and for protein phosphorylation as well as synthesis (14), while intermyofibrillar organelles are involved in the maintenance of muscle contraction and relaxation by contributing to regulate Ca^{++} concentration within the

Figure 1: Electron microscopic picture of intermyofibrillar (arrow) and subsarcolemmal mitochondria (arrowheads) from a human muscle biopsy stained according to conventional procedures. Bar: 0.5 μm .

sarcoplasm (15). Hence, our observations include the ultrastructural features of two discrete mitochondrial populations which have different workloads (16, 17), but are complementary and functionally-correlated.

Since stimulation-driven augmentation of the number of mitochondria in a given cell is accomplished through increase in size, elongation and final splitting of the existing mitochondria into smaller organelles, quantitative estimation of MAA, Dmax and Plei, while providing information on discrete aspects of mitochondrial structural dynamics, constitutes a reliable index of the muscle mitochondria morphofunctional adaptive response to age. Our present findings document an age-related modulation of the ultrastructural features of subsarcolemmal and intermyofibrillar mitochondria. Namely, the decreasing trends of MAA and Dmax in young and middle-aged patients are balanced by the increasing trend of the mitochondrial pleomorphism. With specific reference to Plei, a high value of this parameter may witness consistent restructuring processes as it may occur in mitochondrial degeneration, as a result of mitochondrial fusion, e.g. in conditions of excessive oxidative stress (18, 19). The distribution of Plei values, reported in Figures 4 and 5, show that in the adult and old patients the percent of more pleomorphic organelles increases and this lends further support to hypothesis that there is dynamic remodelling of mitochondrial ultrastructure throughout life.

Figure 2: Mitochondrial average area (MAA), longer diameter (Dmax) and pleomorphic index (Plei) of muscle subsarcolemmal mitochondria. No significant difference due to age can be seen, but MAA and Dmax clearly show a trend to decrease, while Plei appears to increase with advancing age.

In our opinion, the absence of significantly detectable age-related changes in mitochondrial ultrastructure may be due to the several factors playing a role in the age-related decline of muscle performances as well as to the very dynamic condition of muscle tissue (16, 20-22) documented also in old sedentary subjects (12). Muscle aging is a complex process affected by a marked variability due to multiple individual determinants including: muscle disuse, malnutrition and the physiological decline of cardiovascular, nervous and locomotion systems (2, 23). All these causal events contribute to the macroscopic, physiological and incessant muscle atrophy occurring in aging (also referred to as sarcopenia) which is not always associated with a significant decrease of the quality of muscle performances (24) because of the plastic condition of the muscle tissue reported even in aging (5, 6, 25). The recently introduced molecular biology techniques have clearly documented an increase of mitochondrial DNA (mtDNA) deletions in the rodent and primate skeletal muscle with aging (26, 27). At present, it can not be stated whether they are so abundant to cause significant changes, thus mitochondrial dysfunction has been proposed to occur when the load of mutant mtDNA exceeds a tissue-specific threshold (28). In this context, the organelles' metabolic competence (i.e. their capacity to

Figure 3: MAA, Dmax and Plei of muscle intermyofibrillar mitochondria. The same pattern of age-related changes shown for subsarcolemmal organelles is clearly apparent.

Figure 4: Plei percent distribution of subsarcolemmal mitochondria. Higher percentages of less pleomorphic organelles can be seen in younger patients. The higher the pleomorphic index (Plei), more convoluted and distorted the mitochondria.

provide adequate amounts of ATP) is continuously challenged by environmental energy demands and, as a consequence, the mitochondrial populations must adapt their ultrastructural features to give the proper response. As an interpretation of the present findings, we propose that with advancing age, muscle atrophy due to fibre loss consistently involves extensive mitochondrial deterioration (29). Under the stimulation of an increased workload,

Figure 5: Plei percent distribution of intermyofibrillar mitochondria. Although to a lesser extent than for subsarcolemmal organelles, higher percentages of less pleomorphic mitochondria are evident in the youngest group of patients.

it may be suggested that the organelles of the surviving fibres tend to enlarge their size as an adaptive process to respond to an unfavourable environment. It has been recently reported that, in cultured cells exposed to an increased oxidative stress, mitochondria become enlarged and decrease the rate of oxygen consumption, with the final outcome of a reduction in ATP production (30). We are aware of the speculative character of this assumption, nevertheless, muscle mitochondrial populations are documented to adapt their ultrastructural features to the changing volume of muscle mass (31, 32).

EXPERIMENTAL PROCEDURES

Patients

Fifteen, physically active men undergoing surgical interventions were enrolled in the present investigation. They were divided into 3 age groups, each consisting of 5 patients: young (25.0 ± 4.4 years), middle-aged (50.4 ± 7.5 years) and old (75.5 ± 3.9 years) subjects. On the basis of workload similarities, the present morphometric analyses were carried out either on vastus lateralis (VL) or on anterior tibialis (AT) muscles. In details, each age group was composed by 3 VL and 2 AT samples, respectively.

Tissue Preparation and Measurements

Fresh muscle samples were processed according to conventional electron microscopic procedures. Following fixation in 0.1 M cacodylate (pH 7.4) buffered 2% formaldehyde + 5% glutaraldehyde for 24 hours, the muscle pieces were postfixed in 1% osmium tetroxide + 1.5% potassium ferrocyanide for 1 hour. Block staining was carried out by 1 hour incubation of the samples in 1% uranyl acetate dissolved in 0.05 M sodium maleate (pH 5.2). Dehydration in ethanol was followed by embedding in Araldite resin. The tissue sections were counterstained by 1 minute immersion in lead citrate before examination at the electron microscope.

Quantitative analyses were performed on coded samples unknown to the operator. Systematic random sampling was adopted as criterion to select the organelles to be analysed by our computer program. Namely, starting from a fixed position of the electron microscope specimen holder, a random programmed path was run and mitochondrial scoring was stopped at 100 intermyofibrillar as well as subsarcolemmal or-

ganelles/patient. By a TV camera directly connected with the electron microscope, the mitochondrial pictures were recorded on our image analysis system (Kontron KS 300) and their perimeter was manually drawn by means of the computer mouse. The measurements were stored on different files according to the patient's code. The mitochondrial average area (MAA), longer diameter (Dmax) and the ratio perimeter to area, as a pleomorphism index (Plei), were the parameters measured in each patient. Statistical comparisons were performed by analysis of variance (ANOVA) followed by the Student-Neuman-Keuls test.

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ABBREVIATIONS

ANOVA = analysis of variance; **AT** = anterior tibialis; **ATP** = Adenosinetriphosphate; **Dmax** = mitochondrial longer diameter; **MAA** = mitochondrial average area; **mtDNA** = mitochondrial DNA; **Plei** = mitochondrial pleomorphic index; **VL** = vastus lateralis

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