Supplementary information, Data S1

Supplementary methods

Mitochondrial DNA analysis

Mitochondrial DNA (mitoDNA) content was analysed according to a previously described method [1]. Mitochondria were isolated from fresh quadriceps femoris muscles, lysed in 10 mM Tris-HCl (pH 8.0), 20 mM EDTA and 0.5% Triton X-100 (pH 8.0), and occasionally shaken while on ice for 30 min. The mitoDNA was extracted with a mixture of phenol and chloroform, one-tenth volume of 5 mM NaCl was added to the solution and mitoDNA was then precipitated overnight by adding an equal volume of isopropanol. After overnight precipitation at –20°C, mitoDNA was pelletted by centrifugation, resuspended in sterile water, and treated with 50 μg/ml RNase. An aliquot of mitoDNA was loaded on ethidium bromide-stained agarose gel (1.2%) and analysed using the Gel Doc 2000 Imager (Bio-Rad, USA). The mitoDNA levels were determined from the signal intensity normalized to the protein content of muscle from each group. Each sample was also measured by UV absorption at 260 nm using a spectrophotometer.

Fiber type determination

Muscle fiber typing was performed at room temperature using metachromatic dye–ATPase methods. The sections were pre-incubated in solution (pH 4.5) containing 50 mM sodium acetate and 30 mM sodium barbital for 5 min, rinse one time with deionized water and then incubated for 45 min in 20 mM sodium barbital, pH 9.5, containing 9 mM $CaCl_2$ and 2.7 mM ATP. Then the sections were rinsed in 3 changes of 1% $CaCl_2$ (2 min each). For toluidine blue staining [2-3], the sections were stained with 0.5% toluodine blue for 30-60 sec. For $(NH_4)_2S$ staining [4], the sections were first immersed in 2% $CoCl_2$ for 5 min, washed with 3 changes of deionized water and then stained with 1% $(NH_4)_2S$ for 30-60 sec. The sections were then washed with several changes of tap water, dehydrated with ethanol, cleared in xylene, and mounted in balsam. Type I fibers were in dark staining and type II in light staining.

References

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