

Absence of Relationship between the Level of Electron Transport Chain Activities and Aging in Human Skeletal Muscle

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We investigated the relationship between age and respiratory chain function of skeletal muscle mitochondria in 132 control individuals (15 to 95 years old). Muscle mitochondria were studied polarographically and spectrophotometrically. By regression analysis, we found a significant inverse correlation between age and oxygen uptake linked to substrate oxidation (succinate, glutamate-malate or ascorbate-TMPD). However, this significance disappeared after including physical activity and tobacco consumption as confounding variables in a multivariate statistical analysis. Similarly, the activity of respiratory chain complexes individually measured did not decline with age. It therefore appears that respiratory chain activity in human skeletal muscle mitochondria is substantially undamaged during the aging process. © 1996 Academic Press, Inc.

Aging is a biological process that is characterized by a decline in numerous physiological functions. Because mitochondria constitute the major source of ATP utilized by the cell, it has been suggested that this impairment may be secondary to a decrease in mitochondrial respiratory chain (RC) activities (1,2). Furthermore, the worldwide use of PCR detection of mitochondrial DNA (mtDNA) mutations revealed the presence of traces of mutant mtDNA in specific tissues of aged people (3,4). Although the amount of this mutant mtDNA was always far from being sufficient to cause the impairment of the respiratory chain in tissue homogenates or mitochondrial suspensions found in pathological cases (5), a new burst of research was focused on this issue. In keeping with this, we and others have claimed to find an age-related decrease of respiratory chain complex activities in human skeletal muscle (6,7,8,9). On the other hand, other recent studies suggest that age *per se* does not induce any significant RC decrease (10,11).

In the view of such discrepant conclusions, we decided to reinvestigate the relationship between age and RC activity in the particular case of human skeletal muscle. The contribution of two potential confounding variables, physical activity and tobacco consumption (10-13), was assessed. Using quite standardized methods currently used for the screening of respiratory chain deficiencies in humans (14), we studied the mitochondria isolated from skeletal muscle obtained from a large number of individuals: 132 patients from 15 to 95 years old. We conclude that the RC activity in human skeletal muscle is substantially unaffected during aging process.

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Abbreviations: RC, respiratory chain; RCR, respiratory control ration; State 3 and state 4, rates of oxygen uptake in the presence and after phosphorylation of ADP, respectively, TMPD, tetramethyl-*p*-phenylenediamine; ADP/O adenosine diphosphate/oxygen.

MATERIALS AND METHODS

Materials. The study included a total of 132 healthy adult humans who were subjected to orthopaedical surgical procedures for traumatic fractures of the femur. Specimens of quadriceps muscle (1-2 g) were obtained at the beginning of surgery and were immediately processed. All individuals gave informed consent for the procedure. Individuals were requested about their physical activity (normal/subnormal) and tobacco consumption (yes/no).

Mitochondrial studies. Muscle mitochondria were isolated as described (14). The oxygen utilization was measured polarographically with a Clark oxygen electrode in a water jacketed cell at 30°C (15). Protein concentration was determined by the Bradford method (16). The measurement of the activity of the individual complexes of the respiratory chain was carried out spectrophotometrically as described (14).

Statistical analysis. The data were analyzed using SPSS statistical software. The potential association between age

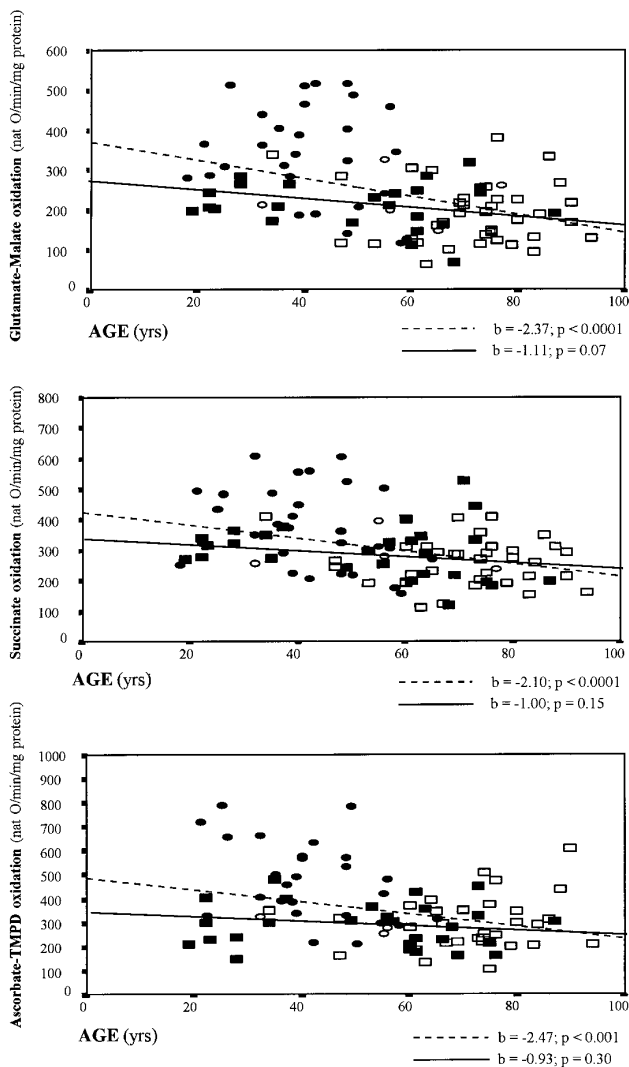


FIG. 1. Statistical analysis of the relationship between State 3 rates of glutamate-malate, succinate or ascorbate-TMPD oxidation in human skeletal muscle mitochondria and age. Oxidation rates were polarographically measured as described (14). The continuous line represents the relationship between age and RC activity once controlled for confounding variables physical activity and tobacco consumption. Dotted line figures a simple liner regression analysis. Solid dots: smokers with normal physical activity; empty dots: smokers with limited physical activity; solid squares: non-smokers with normal physical activity; empty squares: non-smokers with limited physical activity.

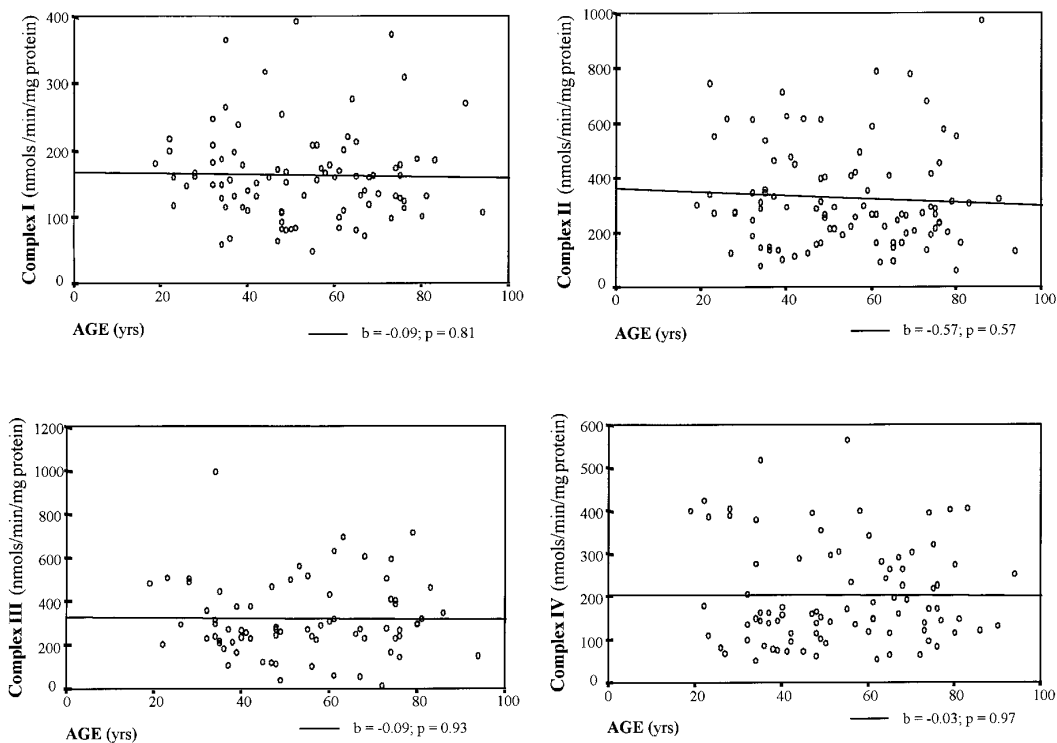


FIG. 2. Statistical analysis of the relationship between individually measured respiratory chain complex activities in human skeletal muscle mitochondria and age. Complex activities were spectrophotometrically measured using standard procedures (14).

and all variables was studied using simple and multiple linear regression analysis. P values <0.05 were considered significant.

RESULTS

In an attempt to uncover a potential relationship between age and RC activity, the rates of substrate oxidation and phosphorylation parameters (ADP/O ratios and RCR) associated to substrate oxidation were performed on mitochondria isolated from skeletal muscle obtained from 132 individuals, the largest cohort ever considered in such a study. Simple regression analysis showed significant inverse correlations between age and State 3 oxidation rates using glutamate-malate ($b = -2.37$, $p < 0.0001$), succinate ($b = -2.10$, $p < 0.0001$), and ascorbate-TMPD ($b = -2.48$, $p = 0.004$) as substrates (Fig. 1, dotted lines). But when potentially confounding variables, such as physical activity (11,12) and tobacco consumption (13), were included in a model of multiple linear regression the previously observed inverse correlation between age and the substrate oxidation rates disappeared (Fig. 1, continuous lines). No differences were found in State 4 rates and RCR ratios. Similarly, ADP/O ratios, taken as a rough index of the mitochondrial phosphorylating ability, did not show any difference with age, suggesting that ATP synthesis ability of the mitochondria did not significantly change with increasing age. It is noteworthy that identical results were obtained when discriminating between women and men.

We next examined the activities of the individual RC complexes in the same group of 132 individuals. Similarly, we did not find any significant correlation between age and RC complex activities, whatever the complex considered (Fig. 2).

DISCUSSION

The above results obtained on a large number of individuals did not reveal any inverse correlation between age and RC activities in human skeletal muscle. Furthermore, when considering potentially confounding variables (physical activity and tobacco consumption), the apparent inverse correlation between age and substrate oxidation rates found in simple linear regression analysis disappeared.

We and others have previously reported such an apparent inverse correlation between age and various RC activities (6-9). However, these results were obtained on much smaller number of individuals and did not consider any potentially confounding variables. Among these latter factors some have already been reported to influence mitochondrial function in skeletal muscle. In man, physical training and exercise have in particular been proved to largely control the level of RC activity in skeletal muscle mitochondria (12). Accordingly, two recent studies (10,11) have established that physical activity is indeed an important factor to consider when studying relationship between age and RC activity in human skeletal muscle. Obviously, the fact that no differences could be observed between sex groups does not rule out the incidence of physical exercise on the relationship between age and RC activity, as previously advocated by others (8).

In view of the present results, it is also noteworthy that the slight increase with age of mitochondrial DNA alterations (mutations or deletions) reported by some authors in skeletal muscle (3,4) does not appear to have any functional incidence on the mitochondrial respiratory chain activity.

As a conclusion, it appears that, as stated ten years ago by Hansford (17) in an impressive review on the bioenergetics in aging in mammals, "mitochondria are substantially undamaged biochemically with aging. . . ." at least in human skeletal muscle.

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