ELSEVIER

Biochimica et Biophysica Acta 1228 (1995) 43–50

BBA Biochimica et Biophysica Acta

Enzymatic activities of mitochondrial respiratory complexes from children muscular biopsies. Age-related evolutions

Etienne Lefai [a], Anne Terrier-Cayre [a], Annie Vincent [a], Odile Boespflug-Tanguy [b], Alain Tanguy [c], Serge Alziari [a,*]

[a] *Laboratoire de biochimie, ERS CNRS 63, Université Blaise Pascal, 63177, Aubière Cédex, France*
[b] *Unité INSERM U.384, Faculté de Médecine, Place Henri Dunant, 63000, Clermont-Ferrand, France*
[c] *Service de Chirurgie Infantile, CHU, Hotel-Dieu, 63000, Clermont-Ferrand, France*

Received 21 June 1994; revised 23 September 1994; accepted 29 September 1994

Abstract

Measurements were performed to determine maximum enzymatic activities of citrate synthetase and respiratory complexes I, III, and IV of mitochondria obtained from muscular biopsies in control children. The significant number of determinations carried out (43 different biopsies in controls aged 3.8 to 19.1 years) permits the formulation of a table of statistically validated reference values for these activities. These values are independent of sex of the controls, and of the studied muscles. Citrate synthetase activity, which remains stable in this age range, thus constitutes a good internal indicator of mitochondrial activity. Complexes I and III manifest activity which does not vary with age. On the other hand, cytochrome oxidase activity shows a highly significant decrease in this age group. This decrease may be correlated with qualitative changes (subunits VIa and VIIa) in composition of this complex.

Keywords: Muscular biopsy; Mitochondrion; Citrate synthetase; Respiratory complex; Cytochrome-*c* oxidase; (Human muscle)

1. Introduction

Severe pathologies with predominantly muscular and nervous system involvement have recently been correlated with mitochondrial dysfunction ([1,2] for review). The central role played by mitochondria in energetic equilibrium of the cell accounts for the fact that any structural or functional mitochondrial damage has severe consequences on the concerned tissues or organism.

Although major pathologies with a characterized mitochondrial correlation have been well described [3,4], changes in the oxidative metabolism may be harder to identify where they provoke only a limited decrease in energetic capacity. In the child, these more discreet energetic deficiencies may have consequences on motor or psychological development.

Studies of these pathologies requires reliable reference values for respiratory complex activities in muscular biopsies derived from children free of muscular pathology. Values available in the literature generally correspond to a limited number of control measurements which cannot be readily exploited as reference values, and there are no studies of possible evolution of these enzymatic activities as a function of age.

The results presented in this publication bear on a substantial number of measurements ($n = 43$), compatible with statistical analysis, thus allowing reference values to be proposed for the activities of citrate synthetase and respiratory complexes I, III and IV.

The good age distribution of tested controls (3.8 to 19.1) allows possible variations in these activities to be studied for this age group. We propose various hypotheses to account for our results.

2. Materials and methods

2.1. Biopsy origins

Muscular biopsies were performed during orthopaedic operations, in the paediatric surgery department of the

* Corresponding author. Fax: +33 73 407042.

SSDI 0005-2728(94)00157-X

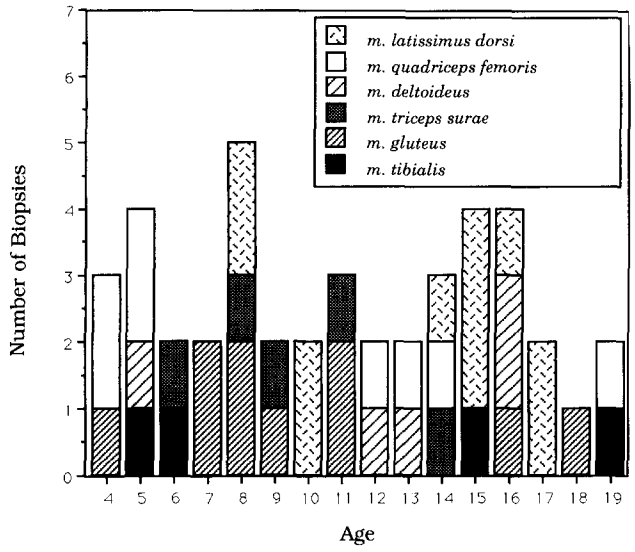

Fig. 1. Distribution of the 43 studied biopsies with age and muscular type. Inset indicate muscular origin.

Hôtel Dieu in Clermont-Ferrand (Authorization from the Consultative Committee for the Protection of Persons Undergoing Biomedical Research, Auvergne Region).

The muscles sampled were those of the scapular belt, musculus deltoideus ($n = 5$); of the pelvic belt, musculus gluteus ($n = 10$), of the limbs, musculus quadriceps femoris ($n = 8$), musculus triceps surae ($n = 5$) and musculus tibialis ($n = 4$); and of the paravertebral muscles, musculus latissimus dorsi ($n = 11$).

None of the muscles studied presented any anomaly in terms of structure or fibre distribution, and none of the children from whom specimens were taken presented disorders imputable to mitochondria. The reasons for surgery were most often injury or congenital malformations.

Age of the studied children ranged between 3.8 and 19.1 years (mean age = 11 years), with age distribution as described in Fig. 1.

2.2. Treatment of the muscle specimen

Immediately after removal, the specimen was cut into fragments for the various analyses. A 200 mg fragment was placed in 4°C buffer (210 mM mannitol, 70 mM sucrose, 50 mM Tris, 10 mM EDTA, and 0.5% bovine serum albumin), then used immediately for the various measurements of mitochondrial enzymatic activity. The other fragments were frozen at −190°C, then stored at −80°C for molecular biological analysis.

Obtention of the total cell extract

The 200 mg fragment was cut with fine scissors then incubated for 30 min in the presence of trypsin (0.5 mg/g of tissue). After rinsing, the muscle tissue was ground in the conical Potter grinder, then centrifuged ($700 \times g$, 10 min) and filtered. The protein concentration of the total cell extract thus obtained was determined.

Obtention of the mitochondrial fraction

The total cell extract was centrifuged twice ($7000 \times g$, 10 min), the pellet taken up with buffer (225 mM mannitol, 75 mM sucrose, 10 mM Tris, 1 mM EDTA) and washed by centrifugation ($700 \times g$, 5 min). After further centrifugation at $7000 \times g$, the mitochondrial pellet was taken up with a minimum volume of the same buffer [5].

Yield was calculated as the ratio of protein derived from the mitochondrial fraction over weight of the biopsy specimen, and expressed in milligrams of protein per gram of tissue.

2.3. Enzymatic assays

Enzymatic assays were performed on the total cell extract and mitochondrial fraction in the DU8 Beckman spectrophotometer, after initial preparation of the mitochondrial fraction to make 1 mg/ml.

Citrate synthetase activity was measured using the technique described by Shepherd and Garland [6], before and after six 1-s sonication cycles. By calculating the citrate synthetase activity ratio for non-sonicated and sonicated fractions, expressed as a percentage, it is possible to evaluate mitochondrial integrity in the observed fraction, citrate synthetase being specifically intramitochondrial.

Assay of complexes I (NADH-ubiquinone reductase), III (ubiquinol cytochrome-*c* reductase), and IV (cytochrome-*c* oxidase) was performed after sonication in keeping with the techniques described by Hatefi and Errede [7–9].

For all measurements of enzymatic activity, each assay was performed in triplicate and the mean was calculated.

2.4. Statistical analysis

For each enzymatic activity, calculation of the mean and the standard deviation was performed for the sum of the values obtained.

Analysis of variance was performed during comparison of the values obtained for each muscle, results being assessed with reference to the Snedecor tables.

A comparison of means allowed analysis of groups per sex for each assay (Student's *t*-test).

The activity–age correlation was calculated to obtain the equation for the regression curve, the value of the correlation coefficient, and the statistical significance of correlation in keeping with the Fisher and Yates table.

Results were validated as non-significant if $P > 0.05$, significant if $0.01 < P < 0.05$, very significant if $0.001 < P < 0.01$, and highly significant if $P < 0.001$.

3. Results

3.1. Sampling

This study involved examination of 43 muscular biopsies derived from six different muscles (see Section 2). The biopsies derived from children of both sexes and different ages. Distribution of the muscle specimens in the various age groups is shown in Fig. 1. The different muscles are homogeneously distributed throughout the age groups, since analysis of variance for the muscle groups shows no significant age difference ($P = 0.44$).

In our sample population, females ($n = 23$) were on average younger than males ($n = 20$) (9.6 years compared with 12.9 years; $P = 0.015$), even if distribution covered a similar range (from 4.7 to 18.8 years for males, and from 3.8 to 19.1 years for females).

As it has been described [10], enzymatic activities of mitochondrial respiratory complexes could be altered by storage at low temperature. To avoid decrease in enzymatic activities, for complex IV in particular, all assays were performed on fresh muscular tissue, with less than thirty minutes from the removal of the biopsy to the mitochondrial extraction.

3.2. Enzymatic activities in mitochondrial fraction

Citrate synthetase

For each biopsy, citrate synthetase activity was measured on the mitochondrial fraction. The comparison of citrate synthetase activity measured before and after sonication allowed assessment of mitochondrial membrane integrity in this fraction. Under the prescribed measurement conditions, integrity was virtually identical regardless of the biopsy, since $81 \pm 8\%$ of citrate synthetase activity was released by sonication.

Analysis of variance of these results for the different muscle groups indicates that there was no significant intergroup difference ($P = 0.43$) (Table 1). Similarly, no significant differences were seen between males and females ($P = 0.40$). These statistical results indicate that, in our sample population, citrate synthetase activity depends neither on the muscle studied nor on sex of the child.

An overall analysis of correlation could thus be performed to identify possible links between activity and age of the child. This correlation was non-significant ($P = 0.31$), providing a mean value which is neither age- nor muscle-dependent (578 ± 253) (Table 1).

Similarly, statistical analysis of yield (milligram of protein from the mitochondrial fraction per gram of muscle) showed no significant difference in terms of muscle ($P = 0.54$) or in terms of sex ($P = 0.67$), and no significant correlation with age ($P = 0.72$). A mean value (0.87 ± 0.43) can thus be proposed (Table 1).

Citrate synthetase activity, measured on the mitochondrial fraction, thus constitutes a reliable reference value, and is independent of the studied biopsy.

Respiratory complexes

Since citrate synthetase activity is a good internal standard, we expressed results in citrate synthetase units (i.e., activity/citrate synthetase activity) for each complex (I, III and IV).

Analysis of variance shows that enzymatic activity of the complexes per citrate synthetase unit does not significantly differ from one muscle to another. For complexes I ($P = 0.16$), III ($P = 0.93$) and IV ($P = 0.38$), activity does not depend on muscular origin of the biopsy (Fig. 2).

Moreover, comparison between males and females does not show significant differences for complexes I ($P = 0.64$), III ($P = 0.25$) and IV ($P = 0.15$), expressed as citrate synthetase units (Fig. 3).

Since citrate synthetase activity is independent of age, we studied the evolution of activity of complexes I, III and IV expressed as citrate synthetase units, as a function of age (Fig. 4).

For complexes I ($P = 0.15$) and III ($P = 0.63$), the analysis of correlation shows no significant evolution of activity with age. Activities of complexes I (0.19 ± 0.12) and III (1.17 ± 0.67) are thus mean values independent of muscle, sex and age of the child, and can therefore be used as reference values (Table 2).

Table 1
Reference values of citrate synthetase activities and yield for the six different muscles studied

Muscle	Citrate synthetase activity (nmol/min per mg)	Yield (mg/g)
m. tibialis ($n = 4$)	529 ± 297	0.64 ± 0.32
m. gluteus ($n = 10$)	593 ± 311	0.77 ± 0.42
m. triceps surae ($n = 5$)	653 ± 267	1.11 ± 0.35
m. deltoideus ($n = 5$)	673 ± 181	0.79 ± 0.26
m. quadriceps femoris ($n = 8$)	656 ± 205	1.00 ± 0.55
m. latissimus dorsi ($n = 11$)	447 ± 228	0.88 ± 0.46
Mean ($n = 43$)	578 ± 253	0.87 ± 0.43

Mitochondria were isolated from biopsies of six different muscles. Yield is expressed in milligrams of mitochondrial proteins per gram of fresh tissue. After a brief period of sonication (6 s), enzymatic activity of citrate synthetase is measured. Data represent mean ± S.D. for n determinations.

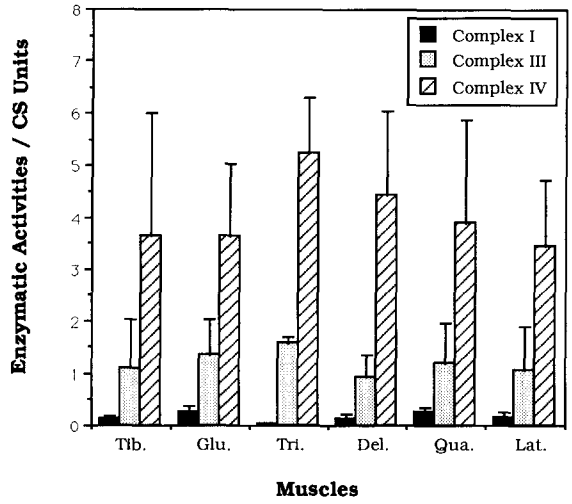

Fig. 2. Enzymatic activities of complexes I, III, and IV in the 6 different studied muscles (Tib., m. tibialis; Glu., m. gluteus; Tri., m. triceps surae; Del., m. deltoideus; Qua., m. quadriceps femoris; and Lat., m. latissimus dorsi). Enzymatic activities were measured on isolated mitochondria as described in Section 2 and are expressed in citrate synthetase units. Figure shows means (bars) and S.D. (vertical lines) for each activity and muscle, except for complex I in m. triceps surae, where only one determination was performed.

On the other hand, the analysis of correlation shows that activity of complex IV (cytochrome oxidase) undergoes a highly significant reduction ($P = 0.0008$) with the age of the child (Fig. 4C). The maximum potential activity of this complexes decreases by about 50% between 4 and 19 years. For this complex, the existing correlation requires attribution of an age-related reference value.

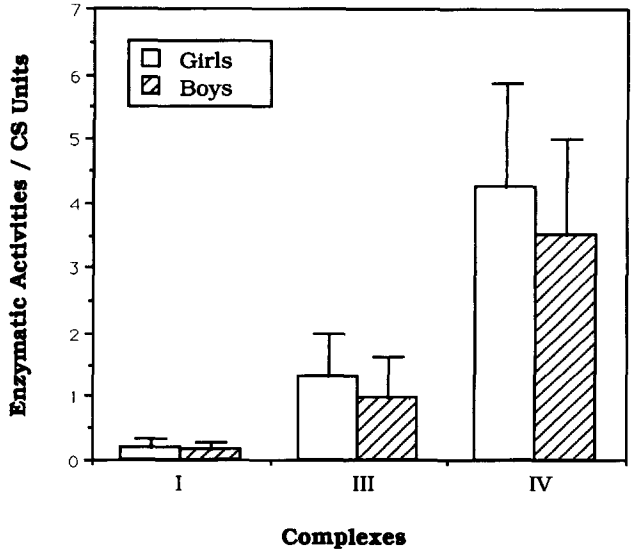

Fig. 3. Enzymatic activities of complexes I, III, and IV in biopsies from girls and boys. Enzymatic activities were measured on isolated mitochondria and are expressed in citrate synthetase units.

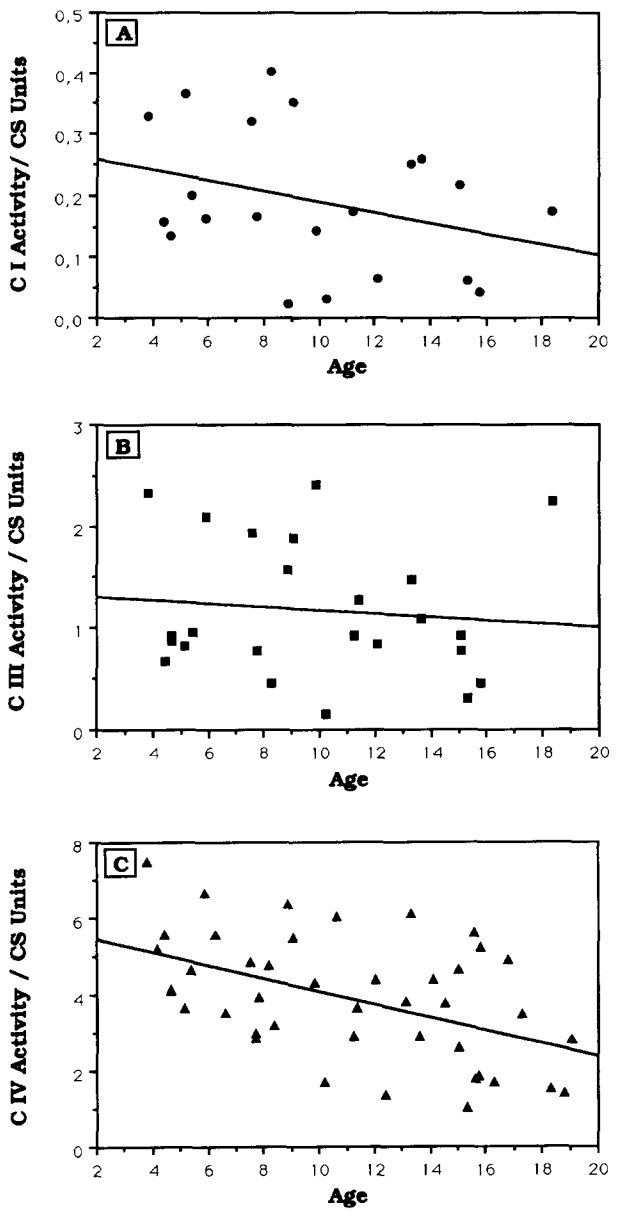

Fig. 4. Evolutions with age of enzymatic activities of complex I (A), III (B) and IV (C). Enzymatic activities were measured on isolated mitochondria and are expressed in citrate synthetase units. Statistical analysis were done and lines represent regression curves.

In light of the statistical significance of the decrease in activity, the mean value of cytochrome oxidase (COX) activity for a given age is the value for this age plotted on the regression curve. The mean value is a linear function of age, such that:

$$\text{COX activity} = 5.7 - 0.17 \times \text{age} \pm 1.41$$

for age ranging between 4 and 19 years, the interval given by the related standard deviation.

These first results allow definition of a set of reference values for mitochondrial enzymatic activity in healthy subjects (Table 2).

Table 2
OXPHOS activities of mitochondria isolated from fresh muscular biopsies

Assay	n	Mean ± S.D.	Range
Citrate synthetase	43	578 ± 253	149–1059
Complex I	21	0.19 ± 0.12	0.02–0.40
Complex III	26	1.17 ± 0.67	0.16–2.42
Complex IV	43	5.7 − 0.17 × age ± 1.41	0.99–7.44

Mitochondria were isolated from fresh muscular biopsies of control children between 3.8 and 19.1 years old. After a brief sonication (6 s), enzymatic activities were measured as describe in Section 2. Citrate synthetase activity is expressed in nmol/min per mg of mitochondrial proteins. Complex activities are expressed in citrate synthetase units. For complex IV, mean is given as an age-related equation. n, Sample size.

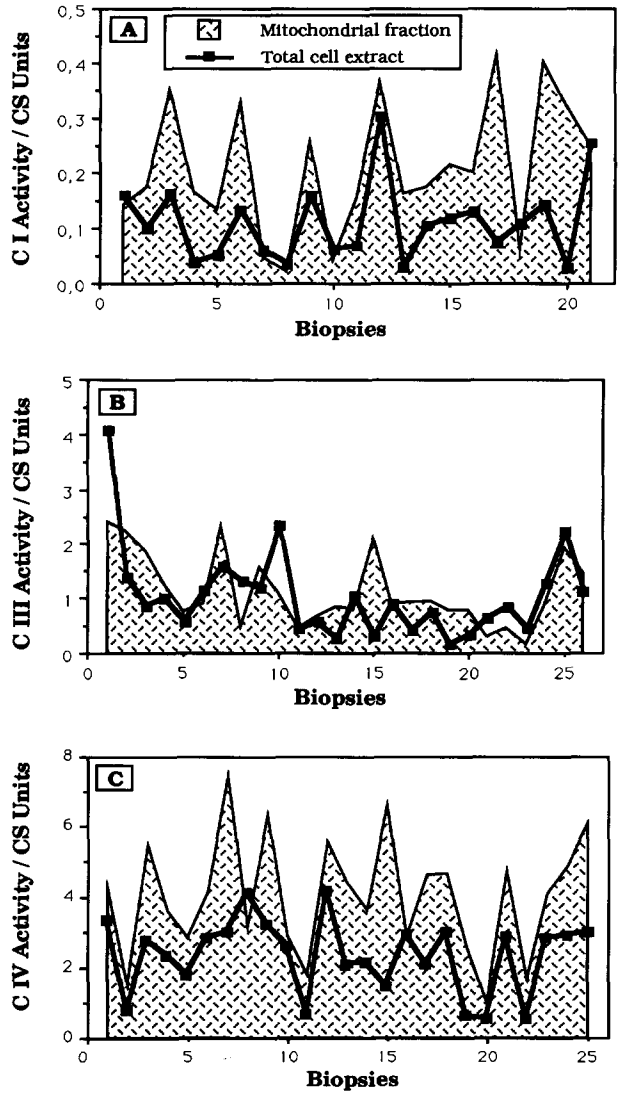

Fig. 5. Comparison of enzymatic activities of complex I (A), III (B) and IV (C) between isolated mitochondria and the total cell extract. For each biopsy, complex enzymatic activities were measured in both fractions after brief sonication (6 s). Enzymatic activities are expressed in citrate synthetase units.

3.3. Enzymatic activities in the total cell extract

Reference values

For certain biopsies, assays of citrate synthetase activity ($n = 26$), complex I ($n = 21$), complex III ($n = 26$) and complex IV ($n = 25$) were performed on both the mitochondrial and total cell extract fractions.

This provides reference values in nmol/min per g tissue for citrate synthetase (5400 ± 2000), complexes I (650 ± 450), III (5900 ± 4500) and IV ($y = 24300 - 1100.0 \times$ age ± 7200), measured on the total cell extract of muscular biopsies from healthy children. Statistical analysis of results obtained for the total cell extract shows exactly the same characteristics as for the mitochondrial fraction: independence of muscular origin of the biopsy, stability of citrate synthetase activity and evolution of complex IV with age ($P = 0.002$).

As for mitochondrial fraction, reference values can be expressed in citrate synthetase units for complexes I (0.12 ± 0.07), III (1.08 ± 0.08) and IV ($y = 3.75 - 0.14 \times$ age ± 0.83).

Comparison of the total cell extract and mitochondrial fraction

Overall, for complexes I, III and IV, the total cell extract yielded lower values than the mitochondrial fraction. The former values represent respectively 63, 92 and 60% of those measured on the mitochondrial fraction (Fig. 5).

The values obtained on the total cell extract showed a highly significant correlation with those obtained on the mitochondrial fraction for complexes I ($P = 0.01$), III ($P = 0.003$) and IV ($P = 0.0002$) (Fig. 5).

This correlation of results of enzymatic assays on total cell extract and mitochondrial fractions permits the analysis of very small biopsies (less than 100 mg), obtention of the mitochondrial fraction no longer being indispensable to the determination of enzymatic activity values for the respiratory chain. Measurement reliability and reproducibility are nevertheless better on the mitochondrial fraction. Where possible, assays should thus be performed on the mitochondrial fraction.

4. Discussion

4.1. Reference values

Validity of the results

The studies presented in this work concern a large number of muscular biopsies (43) from controls aged 3.8 to 19.1 years (Fig. 1). Various muscle specimens (6) were taken during muscular resectioning in the course of orthopaedic surgery.

For all these biopsies, measurements of enzymatic activity of three complexes of the respiratory chain possess-

ing subunits of mitochondrial origin (I, III and IV) were performed on isolated, sonicated mitochondria. Mitochondrial protein yield was reproducible, irrespective of the muscle studied or sex of the child.

Measurements of citrate synthetase activity (specifically intramitochondrial) before and after sonication allow rapid evaluation of quality of the preparation, and of mitochondrial membrane integrity. The increase in activity after sonication (81 ± 8%) is highly reproducible, regardless of the biopsy.

Enzymatic activity of citrate synthetase and complexes I, III and IV, expressed in citrate synthetase units, is independent of the muscle studied and sex of the child. It was thus possible to compare results obtained from different biopsies. Statistical exploitation of these results provides reliable reference values, indispensable for subsequent study of pathological cases.

Reference values

Our results are similar to those in the literature which essentially concern the adult [11,12]. Comparison nevertheless remains limited because of the difference in experimental conditions [13], or in the expression of results [11,14,15].

The wide range of reference values due to individual variability, in particular for complexes I and III inhibit observation of partial deficiencies. Nevertheless, for complex IV, related standard deviation provided a smaller range for a given age. Thus, partial deficiencies for this complex could be observed.

The measurement of citrate synthetase activity on the mitochondrial fraction does not vary with the studied muscle (578 ± 253 nmol/min per mg), apparently indicating very similar mitochondrial metabolic capacities (Krebs cycle) in isolated mitochondrial populations.

Study of the reference values shows potential activity of complex IV to be consistently superior to that of complex III, in turn superior to that of complex I (respective values at 4 years: 5.02, 1.17 and 0.19). The activity of the latter complex may constitute the limit in respiratory chain function.

Electron influx to the complex III level (via ubiquinone), for example, during fatty acid oxidation, would be promoted by higher potential activity of this part of the respiratory chain (III + IV).

The measured activity is maximum potential activity, which may differ from consumed activity during respiratory chain function [16]. Although this function (and relations between the different complexes) can be studied by polarography on isolated mitochondria [11,17,18], such analysis calls for substantial quantities of material (biopsy of 300 to 500 mg). Analysis via isolated fibre [19] requires less material, but is still very difficult. Another approach, currently being developed in the laboratory, is that of the measurement of ATP synthesis capacity by isolated mitochondria, or measurement of the membrane potential. This requires much smaller quantities of biological material (10 to 20 mg of mitochondrial protein).

Values obtained on the total cell extract are correlated with those obtained on the mitochondrial fraction, and display the same characteristics, since complex IV is seen to evolve with age, and complexes I and III are statistically stable. Expressed in citrate synthetase units, complex III activity is quite similar in both fractions. In contrast, complexes I and IV activities are lower in the total cell extract than in mitochondrial fraction. Increase of citrate synthetase activity after sonication is only about 25% in the total cell extract, showing that this fraction contains a higher proportion of disrupted mitochondria than in mitochondrial fraction (where the increase after sonication is about 80%). One hypothesis is that complexes I and IV in such disrupted mitochondria would be less efficient, and complex III should be less affected by preparation of the total cell extract

The total cell extract allows rapid measurement with very little material, thereby allowing characterization of major deficits [20]. Nevertheless, the more precise measurement provided by isolated mitochondria remains indispensable in the case of less pronounced deficits.

4.2. Evolution with age

The large number of analyzed biopsies and their even distribution over ages ranging between 3.8 to 19.1 years (Fig. 1) allowed us to study possible evolution of the reference values as a function of age:

(i) Citrate synthetase, complexes I and III activities did not show statistically significant variations in the studied age range.

(ii) In contrast, the complex IV showed a selective and highly significant decrease between 3 and 19 years (50%).

This decrease with age has already been reported [21,22], but statistical analysis has not yet been done for the age group we studied.

It may be attributable to various causes: modifications in complex IV enzymatic parameters, modified representation of this complex in the mitochondria (quantitative modification), or variation in its subunit composition (qualitative modification). These three possibilities (a–c) are neither exhaustive nor exclusive.

(a) Kinetic parameters (V_m or K_m) of the complex may be modified by effectors extrinsic to the actual complex, i.e., intramitochondrial or cytosolic effectors of subunits of mitochondrial (catalytic) or nuclear origin. Kadenbach et al. [23] showed that ADP could modulate COX activity by binding to the VIαH subunit. This allosteric effect of ADP is tissue-dependent, but can also be considered as development dependent, since it is exerted on a single subunit VI isoform (see below). Determinations of maximum specific activity of this complex have nevertheless been carried out on isolated and sonicated mitochondria. Under our study

conditions, there is thus no direct influence of cytosolic factors.

The lipid environment of the complex, thus membrane composition, may also play a role. While such a modification under the influence of the thyroid hormone has already been described (for review, see [24]), internal membrane transport systems are involved in the present case. Moreover, membrane composition has not been shown to vary in the studied age group.

(b) The variation in cytochrome oxidase activity may be quantitative: it could translate a relative decrease (per mg of mitochondrial protein) in cytochrome-*c* oxidase. According to results obtained with citrate synthetase, the quantity of mitochondria per gram of muscle seems to remain stable. This decrease would therefore correspond to a decrease in complex IV concentration in the mitochondria alone. Although it cannot be ruled out, such a highly specific variation has not yet been described. Any observed variations in activity tend rather to involve the entire respiratory chain. The hypothesis can be tested by estimation of cytochrome concentration in mitochondria isolated from biopsies corresponding to subjects of different ages. Much material is however required. This approach is not compatible with our experiments (biopsies of very limited mass).

If the decrease in complex IV activity corresponds to a quantitative decrease, concentrations of transcripts of the various mitochondrial or cytosolic genes coding for these subunits should likewise be diminished. Steady-state transcript concentrations can be estimated by Northern blot with specific probes. Certain studies, [25], nevertheless show that there is not always a direct relation between steady-state transcript concentrations and those of the corresponding proteins.

(c) Finally, the variation may be qualitative, i.e., reflect a variation in composition of the complex subunits, and therefore different catalytic capacities as a function of this composition. The three subunits of mitochondrial origin are indispensable to function and are always expressed, apparently in a coordinated manner [26,27]. If such a variation exists, it would therefore involve subunits of nuclear origin [28] and would be age dependent. Two subunits in man, VIa and VIIa, can exist as two isoforms, H and L, first identified in different tissues. Whereas isoform L is dominant in the liver, H is dominant in heart and muscle tissue [29,30]. Recent studies have shown that the VIaL form accounts for 17 to 20% of the transcripts of this subunit in embryonic muscle, but accounts for not more than 2 to 4% in adult muscular fibre [27,31]. Similarly, representation of subunit VIIaL goes from about 40% at the fetal stage to 5% at the adult stage. Taanman's research on relative proportions of these two subunits during myogenic differentiation corroborate these findings. There would thus be a qualitative change or switch for these two subunits between the stipulated stages. This change in cytochrome oxidase composition may occur gradually during motor development. The observed variations, under our measurement conditions, might thus be the result of such changes.

Northern blot studies on isolated RNA fractions from biopsies derived from controls of various ages, using specific probes for these subunits, are currently in progress in our laboratory.

Acknowledgements

This work was supported by grants from the 'Association Française contre les Myopathies (AFM)' and the 'Conseil régional d'Auvergne'. It was also supported by the 'Université Blaise Pascal', the 'Université d'Auvergne' and the CNRS.

References

[1] Holt, I.J., Harding, A.E., Cooper, J.M., Schapira, A.V.H., Toscano, A., Clark, J.B. and Morgan-Hugues, J.A. (1989) Ann. Neurol. 26, 699–708.
[2] Wallace, D.C. (1993) Trends Genet. 9, 128–133.
[3] Wallace, D.C. (1991) in Genes, Brain and Behavior (V.A., M.P.R.a.M., eds.), pp. 101–120, Raven Press, New York.
[4] Dimauro, S. and Moraes, C.T. (1993) Arch. Neurol. 50, 1197–1208.
[5] Sherrat, H.S.A., Watmough, N.J. and Jonhson, K.E.M. (1986) Methods Biochem. Anal. 33, 243–335.
[6] Sheperd, D. and Garland, S. (1969) Methods Enzymol. 13, 11–16.
[7] Hatefi, Y. (1978) Methods Enzymol. 53, 11–14.
[8] Hatefi, Y. (1978) Methods Enzymol. 53, 35–40.
[9] Errede, B., Kamen, M.D. and Hatefy, Y. (1978) Methods Enzymol. 53, 40–47.
[10] Pache, Th. and Reichmann, H. (1990) Enzyme 43, 183–187.
[11] Larsson, N.-G., Tulinius, M.H., Holme, E., Oldfors, A., Andersen, O., Wahlström, J. and Aasly, J. (1992) Am. J. Hum. Genet. 51, 1201–1212.
[12] Zheng, X., Shoffner, J.M., Lott, M.T., Voljavec, A.S., Krawiecki, N.S., Winn, K. and Wallace, D.C. (1989) Neurology 39, 1203–1209.
[13] Wallace, D.C., Zheng, X., Lott, M.T., Shoffner, J.M., Hodge, J.A., Kelley, R.I. and Epstein, C.M. (1988) Cell 55, 601–610.
[14] Figarella-Branger, D., Pellissier, J.F., Scheiner, C., Wernert, F. and Desnuelle, C. (1992) J. Neurol. Sci. 108, 105–113.
[15] Birch-Machin, M.A., Shepherd, I.M., Watmough, N.J., Sherrat, H.S., Bartlett, K., Darley-Usmar, V.M., Milligan, D.W., Welch, R.J., Aynsley-Green, A. and Turnbull, D.M. (1989) Pediatr. Res. 25, 553–559.
[16] Debise, R., Touraille, S., Durand, R. and Alziari, S. (1993) Biochem. Biophys. Res. Commun. 196, 355–362.
[17] Byrne, E. and Trounce, I. (1985) J. Neurol. Sci. 69, 319–333.
[18] Larsson, N.-G., Andersen, O., Holme, E., Oldfors, A. and Wahlström, J. (1991) Ann. Neurol. 30, 701–708.
[19] Letellier, T., Malgat, M., Moretto, B., Parrot-Roulaud, F. and Mazat, J.P. (1992) Pediatr. Res. 32, 17–22.
[20] Van Coster, R.N., De Vivo, D.C., Blake, D., Lombes, A., Barret, R. and DiMauro, S. (1991) Neurology 41, 1815–1821.
[21] Paradies, G., Ruggiero, F.M., Dinoi, P., Petrosillo, G. and Quagliariello, E. (1993) Arch. Biochem. Biophys. 307, 91–95.
[22] Hayashi, J.I., Ohta, S., Kagawa, Y., Kondo, H., Kaneda, H., Yonekawa, H., Takai, D. and Miyabayashi, S. (1994) J. Biol. Chem. 269, 6878–6883.

[23] Anthony, G., Reimann, A. and Kadenbach, B. (1993) Proc. Natl. Acad. Sci. USA 90, 1652–1656.
[24] Soboll, S. (1993) Biochim. Biophys. Acta 1144, 1–16.
[25] Izquierdo, J.M. and Cuezva, J.M. (1993) Biochem. Biophys. Res. Commun. 196, 55–60.
[26] Van den Bogert, C., De Vries, H., Holtrop, M., Muus, P., Dekker, H.L., Van Galen, M.J.M., Bolhuis, P.A. and Taanman, J.W. (1993) Biochim. Biophys. Acta 1144, 177–183.
[27] Bonne, G., Seibel, P., Possekel, S., Marsac, C. and Kadenbach, B. (1993) Eur. J. Biochem. 217, 1099–1107.
[28] Kadenbach, B. and Reimann, A. (1992) in Molecular Mechanism Bioenergetics (Ernster, L., eds.), pp. 241–263, Elsevier, Amsterdam.
[29] Fabrizi, G.M., Sadlock, J., Hirano, M., Mita, S., Koga, Y., Rizzuto, R., Zeviani, M. and Schon, E.A. (1992) Gene 119, 307–312.
[30] Arnaudo, E., Hirano, M., Seelan, R.S., Milatovitch, A., Hsieh, C.-L., Fabrizi, G.M., Grossman, L.I., Francke, U. and Schon, E.A. (1992) Gene 119, 299–305.
[31] Taanman, J.W., Hall, R.E., Tang, C.L., Marusich, M.F., Kennaway, N.G. and Capaldi, R.A. (1993) Biochim. Biophys. Acta 1225, 95–100.