# Brain and muscle energy metabolism studied in vivo by $^{31}$P-magnetic resonance spectroscopy in NARP syndrome

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**Abstract**

**Phosphorus magnetic resonance spectroscopy ($^{31}$P-MRS) was used to study in vivo the energy metabolism of brain and skeletal muscle in two members of an Italian pedigree with NARP syndrome due to a point mutation at bp 8993 of mtDNA. In the youngest patient, a 13 year old girl with retinitis pigmentosa, ataxia, and psychomotor retardation, there was an alteration of brain energy metabolism shown by a decreased phosphocreatine content, increased [ADP] and decreased phosphorylation potential. The energy metabolism of her skeletal muscle was also abnormal, as shown by resting higher inorganic phosphate and lower phosphocreatine concentrations than in normal subjects. Her mother, a 41 year old woman with minimal clinical involvement, showed a milder derangement of brain energy metabolism and normal skeletal muscle. Findings with MRS showed that this point mutation of mtDNA is responsible for a derangement of energy metabolism in skeletal muscle and even more so in the brain.**

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We recently reported an Italian pedigree with NARP syndrome,[1] a neurological disorder described by Holt *et al*[2] and characterised by retinitis pigmentosa, ataxia, and psychomotor retardation. The disease is associated with a mitochondrial DNA (mtDNA) point mutation at bp 8993 causing a substitution of a thymine by a guanine in the gene coding for the subunit 6 of ATPase.[2] The resulting replacement of a leucine with an arginine moiety in the hydrophobic sequence of the protein interferes with the membrane $H^+$ channel formed by subunits 6 and 9 of ATPase.[3] This reduces the rate of oxidative phosphorylation measured in vitro in isolated mitochondria prepared from lymphoblast cell lines from patients with this mutation.[4] An obvious in vivo functional consequence should be a deficit of energy metabolism in the patients with this mtDNA mutation, seemingly with a major involvement of the brain. The real in vivo tissue bioenergetics in NARP syndrome, however, have yet to be established.

Phosphorus magnetic resonance spectroscopy ($^{31}$P-MRS) is the only available non-invasive in vivo method to assess mitochondrial function in the brain[5–7] and other organs[8–10] by measuring the relative intracellular concentrations of phosphocreatine [PCr], adenosine triphosphate [ATP], and inorganic phosphate [Pi]. This method is also very sensitive in detecting minimal defects of cellular oxidative metabolism even in the absence of any symptoms or signs.[6 11–14]

We report here a study performed by $^{31}$P-MRS on brain and skeletal muscle of two members from the above pedigree[1] to assess whether the mtDNA point mutation at bp 8993 found in these patients resulted in a deficient energy metabolism. We also related the degree of mitochondrial functionality in these organs to the percentage of heteroplasmy and clinical involvement.

## Patients and methods

PATIENTS

Figure 1 reports the pedigree of the family from which we studied two of the affected members. The family had no record of other relatives with neuroophthalmic disorders.

Patient II-1, a 41 year old woman, had muscle fatigue, headache, and late mild memory loss. Neurological examination disclosed generalised hypotonia, slight proximal leg weakness, absence of knee and ankle jerks, and a minimal Romberg sign. Ophthalmoscopic examination and electroretinography were normal. An ECG, EEG, brain CT and MRI, EMG, serum creatine kinase and lactate (at rest and after effort), muscle biopsy (absence of ragged-red fibres), and spectrophotometric assay of respiratory chain enzymes (NADH dehydrogenase, succinate

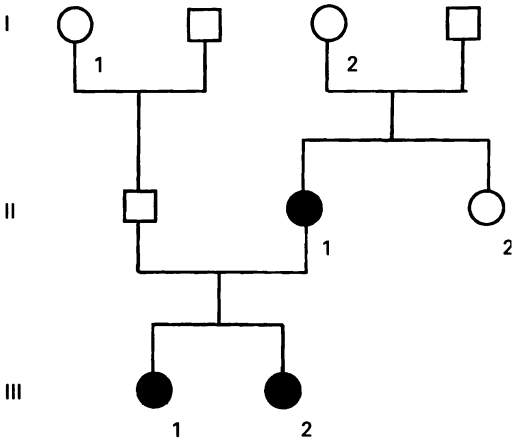


*Figure 1 Pedigree of the family with NARP syndrome. Closed symbols indicate the affected members.*

dehydrogenase, NAD-cytochrome C reductase, cytochrome C oxidase) were also normal. A mutation of mtDNA at bp 8993 was detected, as described,[1] in 10% of mtDNA from leucocytes, skeletal muscle, urinary tract epithelia and hair.

Patient III-1, the 13 year old daughter of patient II-1, had neonatal icterus and has had psychomotor retardation since birth. Neurological examination showed dysarthria, diffuse hypotonia, muscle wasting and weakness, dysmetria, incoordination, gait ataxia with a Romberg sign, and weak tendon jerks. Ophthalmoscopic examination showed a bilateral retinitis pigmentosa, and electroretinography a subnormal photopic response. Brain CT and MRI disclosed cerebellar and brainstem atrophy without cortical cerebral involvement, EEG showed slow background and paroxysmal diffuse activity, and EMG showed neurogenic atrophy and decreased amplitude of sensory evoked potentials in the legs. An ECG was normal, as were serum creatine kinase and lactate (at rest and after effort), muscle biopsy (absence of ragged-red fibres), and spectrophotometric assay of respiratory chain enzymes (NADH dehydrogenase, succinate dehydrogenase, NAD-cytochrome C reductase, cytochrome C oxidase). The 8993 mitochondrial DNA mutation was detected as described[1] in 75% of mtDNA from leucocytes, muscle, urinary tract epithelia, and hair.

Informed consent was obtained from both patients.

### PHOSPHORUS MAGNETIC RESONANCE SPECTROSCOPY ($^{31}$P-MRS)

This was performed by a GE 1·5 Tesla Signa system with a spectroscopy accessory using a surface coil provided by GE and according to the quantification and quality assessment protocols defined by the EEC Concerted Research Project on Tissue Characterisation by MRS and MRI, COMAC-BME II.1.3.[15]

Brain $^{31}$P-MRS was performed on occipital lobes as reported.[12 14] Briefly, spectra were acquired by a GE 1·5 T Signa system with a spectroscopy accessory by a surface coil positioned on the occipital region after imaging the brain. The depth resolved surface coil spectroscopy (DRESS) localisation technique[16] was used to avoid contribution to the signal by neck muscles, skin, and other interposed tissues. The stimulation-response sequence was repeated every five seconds. The flip angle in the selected volume was about 30 degrees, and it was not necessary to introduce any correction for saturation effects due to repetition time. Four hundred free induction decays (FIDs) were accumulated to have a signal to noise ratio of 9 to 12 for $\beta$ ATP. A computerised curve fitting program was used to quantify the individual peaks of the spectrum.[5 12 14] By assuming a cytosolic [ATP] of 3 mM[17] we calculated concentrations of inorganic phosphate [Pi] and phosphocreatine [PCr], [ADP] from the creatine kinase equilibrium,[18] the relative rate of ATP biosynthesis (V/Vmax) from the Michaelis and Menten equation,[18] and the phosphorylation potential.[19]

Muscle $^{31}$P-MRS was performed on the dominant gastrocnemius[20] by the pulse and acquire technique (repetition time of five seconds), firstly at rest, then during isokinetic exercise performed by a pneumatic ergometer,[21] and finally during recovery from exercise. The [Pi] and [PCr] at rest were calculated assuming a cytosolic [ATP] of 8 mM.[22] The rate of postexercise recovery was calculated from the monoexponential equation best fitting the experimental points and reported as time constant (TC).

Intracellular pH was calculated from the shift of Pi from PCr.[23]

### CONTROL SUBJECTS

Control subjects were 20 healthy women aged 14 to 50. No athletes were included in the study. Brain and muscle MRS data did not show any significant age related difference in our control group. Control figures are presented as means (SD). A variable was considered normal when it fell within the range of mean controls (2SD).

*Table 1 Brain (occipital lobes) $^{31}$P-MRS data and mitochondrial function of two patients with NARP syndrome and 20 sex and age matched normal subjects. Examinations were repeated after an interval of 18 months*

| | $^{31}$P-MRS values | | | Calculated mitochondrial values | | |
|---|---|---|---|---|---|---|
| | [PCr] (mM) | [Pi] (mM) | pH | [ADP] (μM) | V/Vmax (%) | PP (mM$^{-1}$) |
| Patient II-1: | | | | | | |
| 1st examination | 3·87 | 1·47 | 7·02 | 35 | 60 | 58 |
| 2nd examination | 3·72 | 1·53 | 7·00 | 36 | 61 | 55 |
| Patient III-1: | | | | | | |
| 1st examination | 3·20 | 1·77 | 7·05 | 50 | 68 | 34 |
| 2nd examination | 3·42 | 1·94 | 7·05 | 45 | 66 | 35 |
| Controls (20) | 4·44 | 1·28 | 7·03 | 28 | 55 | 83 |
| (mean (SD)) | (0·28) | (0·12) | (0·018) | (2·6) | (2·0) | (7·4) |

PP = phosphorylation potential.

*Table 2 $^{31}$P-MRS data of calf muscle at rest and recovering from exercise of two patients with NARP syndrome and 20 normal sex and age matched control subjects*

| | Resting calf muscle | | | | Recovering from exercise | |
|---|---|---|---|---|---|---|
| | [PCr] (mM) | [Pi] (mM) | [Pi]/[PCr] | pH | TC PCr (s) | TC Pi (s) |
| Patient II-1: | | | | | | |
| 1st examination | 26·5 | 4·17 | 0·157 | 7·04 | 37 | 32 |
| 2nd examination | 26·0 | 4·06 | 0·156 | 7·04 | 40 | 35 |
| Patient III-1: | | | | | | |
| 1st examination | 23·5 | 5·12 | 0·218 | 7·06 | | |
| 2nd examination | 23·9 | 5·34 | 0·223 | 7·03 | | |
| Controls (20) | 27·9 | 3·86 | 0·137 | 7·07 | 36 | 34 |
| (mean (SD)) | (1·94) | (0·49) | (0·018) | (0·021) | (3·97) | (3·91) |

Initial recovery is reported as time constant (TC) of the monoexponential equation best fitting the experimental points. Patient III-1 was not able to exercise properly because of mental retardation.

## Results

Both patients were examined by $^{31}$P-MRS twice, as we examined them again 18 months after the first examination (tables 1 and 2).

Figure 2 shows the $^{31}$P-MR spectrum of occipital lobes from patient III-1 compared with an age and sex matched control. The resonance peak of PCr was lower in this patient, and the Pi peak was higher; the $\beta$ ATP peaks were of the same intensity. The calculated [PCr] was very low (more than 3 SD from the

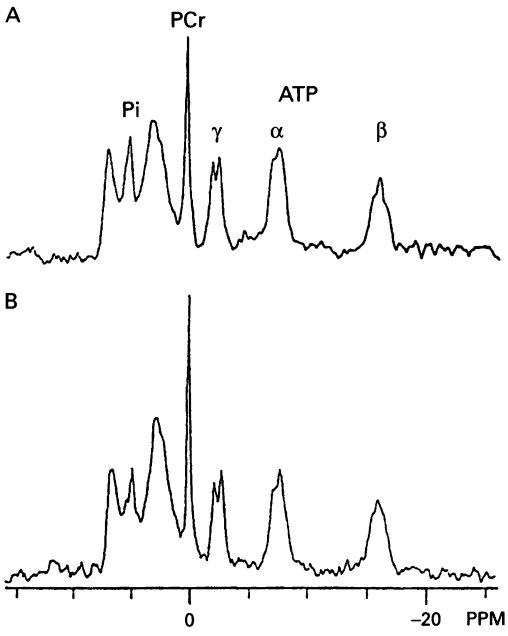


*Figure 2 $^{31}$P-MRS of occipital lobes from patient III-1 (A) compared with an age and sex matched normal volunteer (B). The phosphomonoester peak is located to the left of the Pi peak; the phosphodiester peak is located between the Pi and the PCr peaks.*

control mean) in both examinations, whereas the decrease in [PCr] was less in her mother's brain (subject II-1) in both examinations (table 1). The [Pi] increased in both patients and was higher than 3 SD in the daughter and borderline in her mother (table 1). The [ADP], calculated from the creatine kinase equilibrium,[18] and the percentile value of the maximal rate of ATP biosynthesis (V/Vmax) also increased, with higher values in the daughter than in her mother. The phosphorylation potential was reduced to 41% and 68% of the mean control value in daughter and mother respectively.

Figure 3 shows the spectra of resting calf muscles from patient III-1 (spectrum A) and an age and sex matched control (spectrum B). This patient showed a significant increase in [Pi], a decrease in [PCr], and a remarkable increase in the [Pi]/[PCr] ratio, all variables being more than 2 SD from the control means (table 2). On the other hand, patient II-1 showed MRS data of resting calf muscles within the normal range (table 2). She also showed normal rates of PCr and Pi postexercise recovery as shown by the time constant of the monoexponential equation best fitting the experimental points (table 2). Muscle intracellular pH was within the normal range in both patients (table 2).

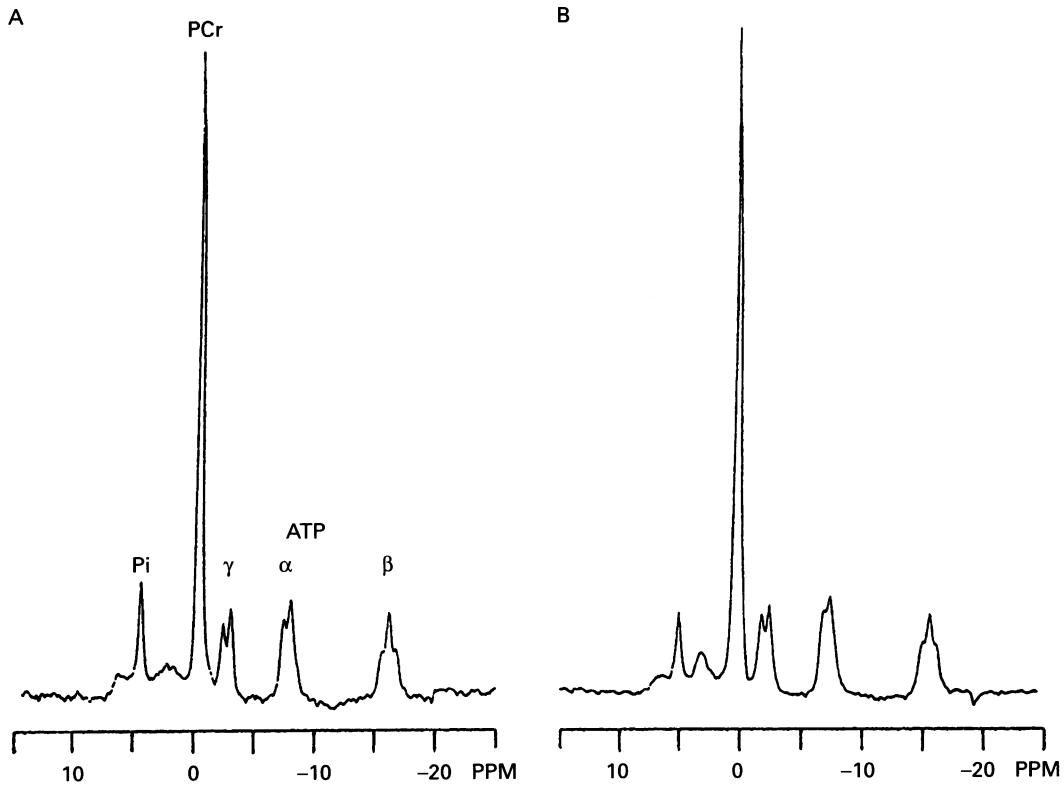


*Figure 3 $^{31}$P-MRS of resting calf muscle from patient III-1 (A) and a sex and age matched normal volunteer (B). Each spectrum consists of 60 scans over a period of five minutes. Resonance assignments as in fig 2.*

## Discussion

We studied only two of the three affected members of the described Italian family with NARP syndrome[1]—namely, the mother (II-1) and her youngest daughter (III-1) (fig 1), as we were unable to perform MRS on the third affected member of the pedigree (III-2, the eldest daughter) because of lack of cooperation due to severe psychomotor retardation. We used neither partial nor total anaesthesia as it could have interfered with brain measurements.

Brain [PCr] was lower and [Pi] higher than the controls in both patients, especially in the daughter, the one with definite signs of CNS involvement. As a consequence, [ADP], calculated from the creatine kinase equilibrium,[18] was also high, the difference from the mean control value being higher in the patient with encephalopathy. It has long been recognised that ADP is the major control molecule of mitochondrial respiration,[24] the relation between the rate of mitochondrial ATP

biosynthesis and [ADP] having the characteristics of a rectangular hyperbola.[18 25] Therefore, a high concentration of cytosolic free ADP indicates that brain cells are operating nearer to the asymptote of the hyperbola of ADP control of respiration and that they are less able to handle any further energy demand. From this it follows that the higher [ADP] found in the brain tissue of patient III-1 indicates a more unstable metabolic condition than that of her mother.

Defective mitochondrial function in both patients was also shown by a higher relative rate of ATP biosynthesis (V/Vmax), which was higher in the daughter than in her mother. We can offer two explanations: either an increased rate of electron transport in functioning mitochondria compensates for the failure of those mitochondria that are unable to satisfy the cell's energy demand, or the theoretical maximal velocity of mitochondrial ATP biosynthesis (Vmax) has a lower value due to the underlying gene defect. As a consequence, the phosphorylation potential, an index of the cells' readily available free energy,[19] was also lower in patient III-1 than in patient II-1, being reduced to 41% and 68% of the mean control value respectively. This finding again reflects a more unstable metabolic condition in patient III-1 than in her mother.

Similar results were also obtained from the skeletal muscle of both patients. Patient III-1 could only be examined at rest due to the severity of her psychomotor retardation; however, $^{31}$P-MRS performed at rest was sufficient to disclose a failure of mitochondrial respiration because of the high degree of defect owing to the presence of 75% mutated mtDNA. A deficient muscle mitochondrial respiration was shown in this patient by a high [Pi] accompanied by a low [PCr] leading to a consequent increase in the [Pi]/[PCr] ratio. The [Pi]/[PCr] ratio is known to represent the cytosolic [ADP],[18 25 26] which is below the sensitivity of in vivo MRS: the higher the [Pi]/[PCr] ratio, the higher the [ADP].

On the contrary, MRS performed on the resting calf muscle of patient II-1 did not show any deficit of mitochondrial respiration. To investigate muscle mitochondria function in this patient more thoroughly we also stressed muscle energy metabolism by exercising her calf muscle inside the magnet to study the kinetics of transitions from the resting state 4 to the activated state 3 of mitochondrial respiration and vice versa. Even under these stressful conditions muscle mitochondria did not show any abnormal functionality, as a normal rate of PCr resynthesis was found during recovery from exercise (table 2), which is known to depend entirely on mitochondrial respiration.[27 28]

In the mother 10% of mutated mtDNA did not cause any deficit of muscle mitochondrial respiration even though the patient complained of muscle fatigue. In view of the ability of $^{31}$P-MRS to disclose small deficits of mitochondrial respiration even in the absence of any symptoms and signs,[6 11-14] muscle fatigue complained of by this patient could be due to subtle neurogenic or CNS involvement.

We do not know the level of heteroplasmy in the brains of our patients. Harding *et al* found a good correlation between the amount of mutant mtDNA in muscle and brain in a foetus with the same point mutation at bp 8993 of mtDNA.[29] If we assume that this is also the case in our patient II-1, the same degree of heteroplasmy (10%) in both brain and skeletal muscle results in a defective energy metabolism in brain and not in muscle because of a major reliance of brain cells on mitochondrial energy production compared with skeletal muscle.[30] On the other hand, we cannot exclude the possibility that a percentage of mutated mtDNA higher in brain than in skeletal muscle allows a mild defect of brain energy metabolism to be detected by $^{31}$P-MRS. From this interpretation it follows that a percentage of mutated mtDNA higher than 10% is needed to determine a mild impairment of brain energy metabolism detectable by $^{31}$P-MRS, whereas a much higher percentage is needed to modify other instrumental indexes. A final understanding of this point—that is, the minimal percentage of mutated mtDNA needed to determine a deficit of energy metabolism detectable by $^{31}$P-MRS, and that which is able to elicit clinical symptoms—needs a larger number of patients with the same gene defect, different levels of heteroplasmy, and different degrees of mitochondrial malfunction.

Variables measured by $^{31}$P-MRS are very sensitive indexes of muscle mitochondrial function either primary or secondary in nature. Hence, our findings, even if not specific for NARP syndrome, are typical of mitochondrial malfunction and overlap the MRS findings of other mitochondrial cytopathies due to different mtDNA defects.[6 11-14]

Our new finding of a defective energy metabolism in both brain and skeletal muscle in patients with NARP syndrome owing to point mutation at bp 8993 of mtDNA confirms our previous findings in larger series of patients with different mitochondrial cytopathies[11 12 14] that energy metabolism may be impaired in the absence of symptoms and signs, and that the phosphorylation potential is lower when encephalopathy is present.

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1 Puddu P, Barboni P, Mantovani V, *et al.* Retinitis pigmentosa, ataxia and mental retardation associated with mitochondrial DNA mutation in an Italian family. *Br J Ophthalmol* 1993;77:84–8.
2 Holt IJ, Harding AE, Petty RKH, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. *Am J Hum Genet* 1990;**46**: 428–33.
3 Tatuch Y, Christodoulou J, Feigenbaum A, *et al.* Heteroplasmic mtDNA mutation (T-> G) at 8993 can cause Leigh disease when the percentage of abnormal mtDNA is high. *Am J Hum Genet* 1992;**50**:852–8.

4 Tatuch Y, Robinson BH. The mitochondrial DNA mutation at 8993 associated with NARP slows the rate of ATP synthesis in isolated lymphoblast mitochondria. *Biochem Biophys Res Commun* 1993;**192**:124–8.
5 Barbiroli B, Montagna P, Cortelli P, *et al.* Complicated migraine studied by phosphorus magnetic resonance spectroscopy. *Cephalalgia* 1990;**10**:263–72.
6 Eleff SM, Barker PB, Blackband SJ, *et al.* Phosphorus magnetic resonance spectroscopy of patients with mitochondrial cytopathies demonstrates decreased levels of brain phosphocreatine. *Ann Neurol* 1990;**27**:626–30.
7 Montagna P, Martinelli P, Cortelli P, Zaniol P, Lugaresi E, Barbiroli B. Brain $^{31}$P-magnetic resonance spectroscopy in mitochondrial cytopathies. *Ann Neurol* 1992;**31**:451–2.
8 Arnold DL, Taylor DJ, Radda GK. Investigation of human mitochondrial myopathies by phosphorus magnetic resonance spectroscopy. *Ann Neurol* 1985;**18**: 189–96.
9 Argov Z, Bank WJ, Maris J, Peterson P, Chance B. Bioenergetic heterogeneity of human mitochondrial myopathies: phosphorus magnetic resonance spectroscopy study. *Neurology* 1987;**37**:257–62.
10 Conway MA, Allis J, Ouwerkerk R, Niioka T, Rajagopalan B, Radda GK. Detection of low phosphocreatine to ATP ratio in failing hypertrophied human myocardium by $^{31}$P magnetic resonance spectroscopy. *Lancet* 1991; **338**:973–6.
11 Cortelli P, Montagna P, Avoni P, *et al.* Leber's hereditary optic neuropathy: genetic, biochemical and phosphorus magnetic resonance spectroscopy study in an Italian family. *Neurology* 1991;**41**:1211–5.
12 Barbiroli B, Montagna P, Cortelli P, *et al.* Abnormal brain and muscle energy metabolism shown by $^{31}$P magnetic resonance spectroscopy in patients affected by migraine with aura. *Neurology* 1992;**42**:1209–14.
13 Barbiroli B, Funicello R, Ferlini A, Montagna P, Zaniol P. Muscle energy metabolism in female DMD/BMD carriers: a $^{31}$P-MR study. *Muscle Nerve* 1992;**15**:344–8.
14 Barbiroli B, Montagna P, Martinelli P, *et al.* Defective brain energy metabolism shown by in vivo 31P MR spectroscopy in 28 patients with mitochondrial cytopathies. *J Cereb Blood Flow Metab* 1993;**13**:469–474.
15 Podo F, Orr JS. *Tissue characterization by magnetic resonance spectroscopy and imaging.* Istituto Superiore di Sanita, Roma: Concerted Research Project, 4th Medical and Health Research Programme of the European Communities, 1992.
16 Bottomly PA, Foster TH, Darrow RD. Depth-resolved surface-coil spectroscopy (DRESS) for in vivo $^{1}$H, $^{31}$P, and $^{13}$C NMR. *J Magn Reson Imaging* 1984;**59**:338–42.
17 Bottomly PA, Hardy CJ. Rapid, reliable in vivo assay of human phosphate metabolites by nuclear magnetic resonance. *Clin Chem* 1989;**59**:392–5.
18 Chance B, Leigh JS, Kent J, *et al.* Multiple controls of oxidative metabolism in living tissues as studied by phosphorus magnetic resonance. *Proc Natl Acad Sci USA* 1986;**83**:9458–62.
19 Veech RL, Lawson JWR, Cornell NW, Krebs HA. Cytosolic phosphorylation potential. *J Biol Chem* 1979;**354**:6538–47.
20 Iotti S, Lodi R, Frassineti C, Zaniol P, Barbiroli B. In vivo assessment of mitochondrial functionality in human gastrocnemius muscle by $^{31}$P-MRS. The role of pH in the evaluation of phosphocreatine and inorganic phosphate recoveries from exercise. *NMR Biomed* 1993;**6**:248–53.
21 Zaniol P, Serafini M, Ferraresi M, *et al.* Muscle $^{31}$P-MR spectroscopy performed routinely in a clinical environment by a whole-body imager/spectrometer. *Physica Medica* 1992;**8**:87-91.
22 Taylor DJ, Styles P, Matthews PM, *et al.* Energetics in human muscle: exercise-induced ATP depletion. *Magn Reson Med* 1986;**3**:44–54.
23 Petroff OAC, Prichard JW, Behar KL, Alger JR, Shulmen T. Cerebral metabolism in hyper and hypocarbia: $^{31}$P and $^{1}$H nuclear magnetic resonance studies. *Magn Reson Med* 1984;**1**:589–93.
24 Chance B, Williams G. Respiratory enzymes in oxidative phosphorylation. I. Kinetics of oxygen utilization. *J Biol Chem* 1955;**217**:383–93.
25 Chance B, Leigh JS Jr, Clark BJ, *et al.* Control of oxidative metabolism and oxygen delivery in human skeletal muscles: a steady-state analysis of the work/energy cost transfer function. *Proc Natl Acad Sci USA* 1985;**82**: 8384–8.
26 Gyulai L, Roth Z, Leigh JS Jr, Chance B. Bioenergetic studies of mitochondrial oxidative phosphorylation using 31-phosphorus NMR. *J Biol Chem* 1985;**260**:3947–54.
27 Taylor DJ, Bore PJ, Gadian DG, Radda GK. Bioenergetics of intact human muscle: a $^{31}$P-NMR study. *Molecular Biology and Medicine* 1983;**1**:77–94.
28 Arnold DL, Matthews PM, Radda GK. Metabolic recovery after exercise and the assessment of mitochondrial function in human skeletal muscle in vivo by means of $^{31}$P-*NMR. Magn Reson Med* 1984;**1**:307–15.
29 Harding AE, Holt IJ, Sweeney MG, Brockington M, Davis MB. Prenatal diagnosis of mitochondrial DNA$^{8993\ T\text{-}G}$ disease. *Am J Hum Genet* 1992;**50**:629–33.
30 Wallace DC, Zheng X, Lott MT, *et al.* Familial mitochondrial encephalomyopathy (MERRF): genetic, pathophysiological, and biochemical characterization of mitochondrial DNA disease. *Cell* 1988;**55**:601–10.