Brain and skeletal muscle bioenergetic failure in familial hypobetalipoproteinaemia

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Abstract

Objective—To determine whether a multisystemic bioenergetic deficit is an underlying feature of familial hypobetalipoproteinaemia.

Methods—Brain and skeletal muscle bioenergetics were studied by in vivo phosphorus MR spectroscopy (^{31}P -MRS) in two neurologically affected members (mother and son) and in one asymptomatic member (daughter) of a kindred with familial hypobetalipoproteinaemia. Plasma concentrations of vitamin E and coenzyme Q_{10} (CoQ_{10}) were also assessed. **Results**—Brain ^{31}P -MRS disclosed in all patients a reduced phosphocreatine (PCr) concentration whereas the calculated ADP concentration was increased. Brain phosphorylation potential was reduced in the members by about 40%. Skeletal muscle was studied at rest in the three members and during aerobic exercise and recovery in the son and daughter. Only the mother showed an impaired mitochondrial function at rest. Both son and daughter showed an increased end exercise ADP concentration whereas the rates of postexercise recovery of PCr and ADP were slow in the daughter. The rate of inorganic phosphate recovery was reduced in both cases. Plasma concentration of vitamin E and CoQ_{10} was below the normal range in all members.

Conclusions—Structural changes in mitochondrial membranes and deficit of vitamin E together with reduced availability of CoQ_{10} can be responsible for the multisystemic bioenergetic deficit. Present findings suggest that CoQ_{10} supplementation may be important in familial hypobetalipoproteinaemia.

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Keywords: familial hypobetalipoproteinaemia; in vivo ^{31}P -MRS; coenzyme Q_{10}

Familial hypobetalipoproteinaemia is an autosomal monogenic disorder in which plasma concentration of apolipoprotein B-100 (APO-B) and low density lipoprotein cholesterol (LDL-C) is abnormally low.¹ This disease can be associated with progressive spinocerebellar ataxia, dementia, peripheral neuropathy, retinitis pigmentosa, fat malabsorption, acantocytosis, and low plasma content of vitamin

E.² Neuromuscular manifestations can be found in heterozygotes, whereas haematological and gastrointestinal disorders are rare.²

The pathogenesis of this disorder is not yet fully understood. However, neurological symptoms and signs and the underlying neuropathological changes have been related to altered composition of cellular membranes due to the deficit of lipoprotein and vitamin E found in these patients^{3,4} and to the inability of the liver to incorporate vitamin E in the very low density lipoprotein and in LDL.⁵ A deficit of vitamin E leads to an excessive lipid peroxidation with production of lipid peroxyl radicals which in turn can result in damage to cell membranes^{3,6} as well as proteins and DNA.^{7,8} Therefore, it is conceivable that by different mechanisms, the damage due to free radicals results in an impairment of mitochondrial functionality and ATP production.⁹⁻¹²

Tissue bioenergetics can be assessed in vivo by phosphorus magnetic resonance spectroscopy (^{31}P -MRS), the only available non-invasive method that gives precise information on the efficiency of ATP production and the extent to which oxidative metabolism meets the bioenergetic needs of cell function.

The aim of this study was to investigate by in vivo ^{31}P -MRS whether a multisystemic defect of tissue bioenergetics is an underlying feature of the neurological disorders found in patients with a deficit of APO-B.

In view of the antioxidant role of vitamin E and the bioenergetic and antioxidant functions of CoQ_{10} ,^{7,13} we also assessed the plasma concentrations of vitamin E and CoQ_{10} .

Patients and methods

PATIENTS AND LABORATORY DATA

Figure 1 shows the family pedigree. Subjects with LDL-C concentration below the fifth percentile of values for age and sex matched local populations¹⁴ were classified as affected by hypobetalipoproteinaemia, according to published criteria.^{1,2,15} Three members of the family with hypobetalipoproteinaemia, two neurologically affected, with LDL-C lower than the fifth percentile (II-5 and III-2), and one asymptomatic, with LDL-C between the fifth and the tenth percentile (III-3), underwent the in vivo study of energy metabolism of both brain and skeletal muscle. The fourth asymptomatic subject (III-5), with hypobetalipoproteinaemia (LDL-C concentration below the fifth percentile), did not consent to MRS examination.

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Figure 1 Pedigree of the family with familial hypobetalipoproteinaemia.

Case II-5 (proband)
This 55 year old woman had a severe bilateral neurosensory hearing deficit which had developed in a few years after the age of 25. From the age of 40 she showed difficulty in walking associated with tremors in the upper and lower limbs, tingling in the hands, and personality changes. At 46 mental deterioration and a pronounced spinocerebellar syndrome with ataxic-spastic gait were found on examination. She worsened slowly and, at 55, developed a sensory-motor polyneuropathy and became bedridden.

Case III-2
The 29 year old son of the above patient had a slow psychomotor development until 15, but no evident cognitive impairment in adulthood. When he was 17 he had a sudden loss of vision in the left eye and a bilateral optic disc oedema, followed by a bilateral atrophy of papilla. His visual acuity was very reduced in the left eye and normal on the right.

Case III-3
The 25 year old daughter of the proband was asymptomatic and normal on neurological examination.

Table 1 Lipid serum concentrations in three subjects studied

Case	Age (y)/ Sex	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	TG (mg/dl)	APO-A1 (mg/dl)	APO-B (mg/dl)
II-5	55/F	127 (< 5)	61 (< 5)	53	64 (< 10)	138	48 (< 5)
III-2	29/M	82 (< 5)	29 (< 5)	45	40 (< 10)	140	47 (< 5)
III-3	25/F	144 (< 5)	81 (< 10)	53	48 (< 10)	151	42 (< 10)

The percentile values of serum lipid concentration are shown in parentheses only when in the bottom decile. Percentiles were determined for age and sex matched population of the same Italian region as the studied kindred.^{14,61}

In all three subjects serum biochemical tests, performed according to published methods,¹⁶ showed a significant decrease in serum content of total cholesterol (TC), LDL-C, triglycerides (TGs), and APO-B, whereas HDL-cholesterol (HDL-C) and APO-A1 concentrations were normal (table 1). Quality control was carried out by the World Health Organisation (WHO) Lipid Research Centre in Prague according to the procedure adopted for lipid clinics participating in the WHO-ERICA projects.¹⁷

The search for point mutations of mtDNA at positions 11 778, 3460, and 14 484, pathogenic for Leber hereditary optic neuropathy,¹⁸ was negative in blood white cells from the proband and her son with bilateral optic atrophy.

Control subjects were 49 healthy volunteers. No athletes were included in the study. Control figures are presented as means (SD). A variable was considered normal when it fell within the range of mean controls $\pm$ 2SD.

Informed consent was obtained in all cases.

PHOSPHORUS MAGNETIC RESONANCE SPECTROSCOPY (³¹P-MRS)

³¹P-MRS was performed with a GE 1.5 Tesla Signa system with a spectroscopy accessory using a surface coil provided by General Electrics and according to the quantification and quality assessment protocols defined by the EEC Concerted Research Project on Tissue Characterisation by MRS and MRI, COMAC-BEM II.1.3.¹⁹

Brain ³¹P-MRS was performed on the occipital lobes as reported.²⁰ Spectra were acquired by a General Electrics 1.5 T Signa system with a spectroscopy accessory by a surface coil positioned on the occipital region after imaging the brain. The DRESS (depth resolved surface coil spectroscopy) localisation technique²¹ 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°, and it was not necessary to introduce any correction for saturation effects due to repetition time. Four hundred free induction decays were accumulated to have a signal to noise ratio of nine to 12 for β -ATP. A computerised curve fitting program was used to quantify the individual peaks of the spectrum. By assuming a cytosolic ATP concentration of 3 mM,²² we calculated inorganic phosphate (Pi) and phosphocreatine (PCr) concentrations, ADP concentration from the creatine kinase equilibrium,²³ and the phosphorylation potential.²⁴ We do not have absolute data on ATP concentration in patients with familial hypobetalipoproteinaemia. However, if ATP concentration were lower in these patients, the calculated ADP concentration would be even higher.

Muscle ³¹P-MRS was performed on the right gastrocnemius²⁵ by the pulse and acquire technique (repetition time of five seconds), at rest, during in magnet aerobic isokinetic exercise,²⁶ and during recovery from exercise.

Figure 2 Upper section: ^{31}P -MRS of occipital lobes from proband (II-5) compared with an age and sex matched normal volunteer. Lower section: ^{31}P -MR spectra of resting calf muscle from the same subject and a sex and age matched normal volunteer. Pi = inorganic phosphate; PCr = phosphocreatine; the phosphomonoester peak is located to the left of the Pi peak; the phosphodiester peak is located between the Pi and the PCr peaks.

Concentrations of Pi and PCr at rest were calculated assuming a cytosolic ATP concentration of 8.2 mM.²⁷ Cytosolic ADP concentration was calculated from the creatine kinase equilibrium.²⁸

The efficiency of mitochondrial ATP production was assessed by measuring the rate of

postexercise ADP and PCr recoveries which are entirely oxidative.^{28,29} The half life of ADP recovery was calculated from the slope of semilogarithmic plots. The rates of PCr resynthesis and of Pi recovery rates were calculated from the monoexponential equation best fitting the experimental points and reported as time constants (TCs).²⁵

Intracellular pH was calculated from the chemical shift of Pi relative to PCr.³⁰ The chemical shift was carefully determined from the centre of the PCr peak to the centre of the Pi peak.

VITAMIN E AND COQ₁₀ DETERMINATION

Plasma vitamin E was estimated by high performance liquid chromatography (HPLC) using a fluorescence detector set at an excitation of 292 nm and an emission of 335 nm.³¹ Plasma CoQ₁₀ was quantified, according to Takada *et al.*,³² with reverse phase HPLC using an Erbasil C 18/M 150 × 4.6 mm column and a mobile phase of propanol/methanol (20:80) with an isocratic 2 ml/min flow; column eluate was monitored at 275 nm in a Varian 2010 instrument.³³ CoQ₁₀ data from patients were compared with the reference range used in our clinical laboratory (0.693 mg/l; mean of 86 reference subjects).

Results

Figure 2 shows the ^{31}P -MR spectra obtained from the occipital lobes of case II-5 and an age and sex matched control. The resonance peak of PCr was reduced in this patient, whereas the β -ATP peaks were of the same intensity. Concentration of PCr was also significantly reduced in the proband's son and daughter (table 2). The asymptomatic daughter (case III-3) showed the lowest brain PCr concentration (table 2). Inorganic phosphate concentration was markedly increased in case III-2 whereas it was within the reference range in case II-5 and in case III-3. The ADP concentration was significantly increased in all three cases, showing the highest concentration in case III-3. The phosphorylation potential was reduced to 63% of the mean control value in all three cases.

Figure 2 also shows the spectra of resting calf muscles from case II-5 and an age and sex matched control. This patient showed a significant increase in Pi concentration whereas both PCr concentration and cytosolic pH were normal (table 3). On the other hand, cases III-2 and III-3 showed MRS data of resting calf muscles within the normal range (table 3). Only cases III-2 and III-3 exercised inside the magnet. In both cases the end exercise PCr concentration was about 50% of the resting content. Ten normal volunteers were asked to exercise to deplete PCr to about the same concentration as the two patients (table 3). Despite the same metabolic activation, both cases showed a much higher end exercise cytosolic pH (table 3). The ADP concentration at the end of exercise was significantly above normal control values (table 3). The rate of ADP postexercise recovery was signifi-

Table 2 Brain (occipital lobes) ^{31}P -MRS data and mitochondrial function of three family members studied and 30 sex and age matched healthy subjects

Case	^{31}P -MRS values			Calculated mitochondrial values	
	PCr (mM)	Pi (mM)	pH	ADP (μM)	Phosphorylation potential (mM^{-1})
II-5	3.65*	1.36	7.06	43*	52*
III-2	3.77*	1.66*	7.02	37*	52*
III-3	3.21*	1.21	7.04	48*	52*
Controls (mean (SD))	4.44 (0.28)	1.28 (0.12)	7.03 (0.018)	28 (2.6)	83 (7.4)

*Values 2 SD or more from the mean of normal controls.

Table 3 ³¹P-MRS data of calf muscle at rest, at the end of exercise, and during the subsequent recovery of three family members studied and 49 normal sex and age matched healthy controls

Case	Rest			End exercise			Recovery			
	PCr (mM)	Pi (mM)	pH	PCr (%)	ADP (μM)	pH	pH _{min}	t ₁ ADP (s)	TC PCr (s)	TC Pi (s)
II-5	30.9	6.24*	7.11	—	—	—	—	—	—	—
III-2	25.6	4.31	7.07	49	100*	6.95	6.78	11.1	28.0	42.9*
III-3	26.5	4.45	7.03	51	90*	6.95	6.79	19.3*	43.7*	49.8*
Controls mean (SD)	27.9† (1.94)	3.86‡ (0.49)	7.07† (0.021)	50.4‡ (2.81)	58‡ (13.5)	6.84‡ (0.07)	6.85§ (0.04)	10.8† (3.62)	29.3¶ (4.57)	30.1¶ (4.98)

*Values 2 SD or more from the normal controls mean.
†49 controls; ‡10 controls who reached an end exercise PCr depletion of 50.4 (2.81)% of the resting value; §10 controls who reached an end exercise pH of 6.95 (0.03); ¶10 controls who reached a minimum pH during recovery of 6.79 (0.09).
Case II-5 was not able to exercise properly because of mental deterioration and motor impairment. TC = Time constant.

Figure 3 Time course of cytosolic pH recovery from exercise in cases III-2 (solid squares) and III-3 (solid circles) compared with 10 sex and age matched controls (open circles) that reached a similar cytosolic pH (6.95 (0.03)) at the end of exercise. Control data are shown as means (SD).

cantly reduced only in case III-3. In view of the influence of the minimum pH after exercise on the rate of PCr and Pi recovery,²⁵ patients were compared with 10 normal controls who reached a similar postexercise minimum pH. These experimental conditions render precise information on the functionality of mitochondria respiration independently of muscle mass, fibre type, and mitochondria density.³⁴ The PCr recovery rate was normal in case III-2 and significantly slow in case III-3. In both cases the rate of postexercise Pi recovery showed a significant reduction (table 3).

Figure 3 illustrates the pattern of pH recovery in cases III-2 and III-3 and in 10 normal controls that reached a similar end exercise intracellular pH. In the above subjects the pH overshooting tended to be faster and of higher degree than controls, although not significant.

The concentrations of plasma vitamin E and CoQ₁₀ were below the normal range in all three subjects (table 4).

Table 4 Plasma coenzyme Q₁₀ and vitamin E concentrations in three subjects studied

Case	Vit E (mg/l)	CoQ ₁₀ (mg/l)
II-5	4.24	0.37
III-2	3.90	0.36
III-3	4.10	0.58
Normal range:		
Male	8.9–18.3	0.6–0.8
Female	9.4–15.0	

Discussion

The main finding of our study was a defective brain and skeletal muscle energy metabolism in two symptomatic relatives (cases II-5 and III-2) that fulfil the clinical and laboratory criteria for familial hypobetalipoproteinaemia and in one symptom free subject of the same kindred (case III-3) with a borderline low value of LDL-C.^{1 2 15} In all subjects we also found a reduced plasma content of CoQ₁₀, implicating CoQ₁₀ in the pathogenesis of the bioenergetic deficit.

Defective brain bioenergetics can be recognised by knowing the concentration of PCr, Pi, and ADP.³⁵ Phosphocreatine was reduced in our three patients, case III-3 showing the lowest value. On the other hand, Pi showed a significant increase only in case III-2 (table 2). This result is difficult to understand as reciprocal alteration in PCr and Pi as a result of defective mitochondrial respiration would be expected, and we do not have any straightforward interpretation to offer. However, it is worthy to note that also in other conditions no reciprocal changes in PCr and Pi have been found.^{36–38}

The ADP concentration was increased in all patients, showing the highest values in the symptom free daughter (case III-3). A high concentration of cytosolic free ADP, that exerts a hyperbolic control on oxidative phosphorylation,³⁹ indicates that brain cells are operating nearer to the asymptote of the hyperbola and that they are less able to handle any further energy demand. The increase in ADP or Pi concentrations resulted in all three patients in a significant reduction of the cytosolic phosphorylation potential, an index of the cell's readily available free energy.²⁴ This indicates an unstable metabolic condition of the brain of all subjects.

A bioenergetic deficit was also detected in the skeletal muscle (table 3). Case II-5 was examined only at rest due to the severity of her mental and motor impairment. Nevertheless, the ³¹P-MRS examination performed at rest was sufficient to disclose a failure of mitochondrial respiration as shown by increased concentration of Pi. A high concentration of Pi indicates a severe impairment of muscle energy metabolism occurring in some disorders with primary and secondary mitochondrial dysfunction.^{40 41} On the contrary, cases III-2 and III-3 showed all resting values within normal range (table 3). However, in cases III-2

and III-3 we were able to investigate the metabolism of skeletal muscle under stressful conditions—that is, during exercise and recovery from exercise. In both cases the end exercise ADP concentration was higher than in controls who reached a similar PCr depletion at the end of exercise. The rise in ADP concentration stimulates mitochondria to operate closer to their maximal activity and may lead to normal mitochondrial ATP production in some neuromuscular disorders.^{42,43} The stimulation of mitochondrial respiration by ADP resulted in a normal rate of mitochondrial ATP production in case III-2, as shown by a normal ADP and PCr recovery, but it could not drive the rate of mitochondrial phosphorylation to the normal range in case III-3 (table 3). The end exercise pH was somewhat higher in both cases than in matched controls that exercised to the same extent. Increased end exercise pH can be due to increased proton efflux or reduced lactate production.⁴⁴ In both patients the proton efflux seems to be reduced—although not significantly—when compared with normal subjects with similar end exercise pH (fig 3). Therefore, a reduced contribution of anaerobic glycolysis is the more likely explanation for high pH at the end of exercise.

Postexercise recovery rate of Pi was slow in both patients compared with a group of normal subjects who reached a similar postexercise minimum pH (table 3). Changes in membrane composition, due to defective plasma transport of cholesterol and triglycerides³ together with the membrane damage due to free radicals,^{4,45} may be responsible for the abnormal transport of both Pi and H⁺. In this light it is worth stressing that a slow rate of Pi and H⁺ transport has been found in patients with Becker dystrophy and carriers⁴⁶ and a slow rate of H⁺ transport has been described in *mdx* mice,⁴⁷ being interpreted as a consequence of membrane malfunction.

Spinocerebellar syndrome with ataxia, peripheral neuropathy, and areflexia, are the main clinical features common to disorders such as abetalipoproteinaemia and hypobetalipoproteinaemia,^{4,45} cystic fibrosis,⁴⁸ cholestatic liver disease,⁴⁹ short bowel syndrome,⁵⁰ and isolated vitamin E deficiency.⁵¹ In these diseases the content of vitamin E is decreased in serum and in peripheral nerves.⁴ Although only the proband of our kindred showed a spinocerebellar syndrome whereas her son showed a bilateral optic atrophy—not described so far in association with hypobetalipoproteinaemia—and her daughter was asymptomatic, in all three cases we found a low plasma content of vitamin E as well as of CoQ₁₀. We do not know the actual content of CoQ₁₀ in the skeletal muscle of our patients. However, it has been shown that the plasma concentration reflects the tissue content of CoQ₁₀.^{52,53} The known functions of CoQ₁₀—that is, the electron transport in the inner mitochondrial membrane and—in its reduced form—antioxidant activity,^{7,13} lead us to hypothesise that the reduced plasma content of CoQ₁₀ might play a part in the pathogenesis

of neurological damage. There is evidence that CoQ₁₀ is necessary for the regeneration of vitamin E from the α -tocopheroxyl radical, whereas ubiquinol does not require vitamin E for its antioxidant activity.⁷ Coenzyme Q₁₀ is synthesised in all tissues by the mevalonate pathway^{7,54} and is transported in the bloodstream mainly bound to LDL, but its tissue redistribution from plasma is not yet established.⁷ It is also known that an increased availability of circulating CoQ₁₀ ameliorates the efficiency of energy metabolism.^{55–57} This suggests that low CoQ₁₀ could be responsible for a reduced efficiency of respiratory chain, or an increased oxidative stress of membranes, or both, possibly leading to neurological disturbances.

The different phenotype reported in apparent primary CoQ₁₀ deficiency⁵⁸ does not exclude a pathogenic relevance of the CoQ₁₀ deficit. In fact, in primary mitochondrial disorders a similar bioenergetic deficit²⁰ and even the same mtDNA mutation may result in different clinical expressions.⁵⁹

We did not find a direct relation between the degree of energy metabolism failure and the clinical picture. In fact, the asymptomatic daughter (case III-3) had the most compromised energy metabolism. This is not a surprising finding as brain bioenergetic impairment has been described in patients affected by mitochondrial myopathy without any brain symptoms.^{20,60} However, case III-3 is also the youngest of the examined patients and the time of exposure to the persistent energy metabolism defect may contribute to the development of neurological impairment.

Our results illustrate that a multisystem impairment of bioenergetics and a reduced rate of muscle Pi transport are biochemical features of familial hypobetalipoproteinaemia. They also show that a bioenergetic derangement can be present in asymptomatic patients. Three main factors may be responsible for the multisystem energy failure found in our patients: (1) structural changes of the inner mitochondrial membrane leading to spatial changes among respiration complexes thus interfering with the diffusion of mobile electron transporters in the mitochondrial inner membrane; (2) deficit of vitamin E leading to increased production of free radicals and consequent damage of mitochondrial membranes, DNA, or respiratory complexes; (3) reduced availability of CoQ₁₀ in the inner mitochondrial membrane causing an impairment of oxidative phosphorylation.

The CoQ₁₀ depletion, found in all the patients, suggests that CoQ₁₀ supplementation may be important to halt further progression or to prevent neurological abnormalities in familial hypobetalipoproteinaemia.

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