Riboflavin-Responsive Metabolic Myopathy with Abnormal Fatty Acid Oxidation and Defective Intramitochondrial Respiratory Complexes

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Abstract

We describe a riboflavin and carnitine responsive lipid storage myopathy in a 36 -year- old male, who presented generalized weakness, muscle carnitine deficiency (11% of control) and decreased beta-oxidation in fresh muscle homogenate. Following riboflavin supplementation (100 mg/die) the patient's condition improved dramatically and recovered was restored.

The fatty acid oxidation defect was associated with a defect in mitochondrial respiratory complexes I, II and IV (42%, 17% and 45% of normal respectively). Flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD) and riboflavin (Rb) were measured in frozen muscle homogenate with a specific and sensitive HPLC method, and their content was comparable to that of healthy controls. This suggests a possible defect in mitochondrial transport of FAD or FMN in this patient.

Key words: riboflavin, carnitine, myopathy, fatty acids, respiratory complexes.

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Riboflavin responsive myopathies are a group of disorders that are of interest to clinical investigators since a therapeutic supplementation might be provided to promptly restore a normal clinical and biochemical status.

Riboflavin responsive disorders are characterized by their therapeutical response from other inborn metabolic errors in mitochondrial chain complexes [18], from defects of fatty acid oxidation [3, 12] and from combined causes [2]. Age at onset ranges from neonatal period to adulthood. The clinical phenotype is usually more severe in pediatric patients whereas adults patients often present muscle weakness [9]. It is believed that in such riboflavin-responsive patients, the primary defect probably lies in riboflavin metabolism or in the metabolism of its coenzymes, FMN and FAD [14].

We here describe an adult male with a riboflavin-responsive severe metabolic myopathy and report on the results of biochemical investigations performed in patient's muscle biopsy before riboflavin treatment.

Material and Methods

Case report

The patient was in good health until 36 years of age (his family history was negative for neuromuscular disorders). He worked as a clerk but when he was off duty he could do agricultural work without difficulty. In March '93 the patient noticed leg weakness, and then experienced a gradual generalized weakness in neck muscle and in upper girdle muscle without pain and cramps or myoglobinuria. In June 93 the patient changed his job (computer clerk in a factory) since he could not hold his head in an erect position. He was hospitalized in November '93 for a severe generalized myopathy, at admission he was virtually tetraplegic and could move only his fingers. The following serum enzymes were elevated: creatine kinase 2700 U/l (normal 50-220 U/l.), lactate dehydrogenase 848 U/l (normal 100-220 U/l), aldolase 21.9 U/l (normal up to 7.6 U/l), glutamic oxalacetic transaminases 313 U/l (normal 7-40 U/l) and pyruvic oxalacetic transaminases 191 U/l (normal 6-40 U/l); uric acid was 10.8 mg/dl (normal 2.2-7.5 mg/dl). In the urine abnormally high ketone body levels were found. Electrocardiography showed tachycardia while the electromyogram was myopathic. Since the patient's masticatory muscles were weak, he had fasted for a long period, with a consequent weight loss of 14 kg. Stools were positive for Stronghiloides Stercolarum, his parasitic infection was treated in February 1993.

Gas-liquid chromatography of urinary organic acids showed a normal pattern in a sample taken two months after an attack of acute ketonuria. Plasma and urine carnitine levels were normal.

The patient was treated with prednisolone 75 mg/die for two months, carnitine 4 g/die and riboflavin 100 mg/die, and his condition improved rapidly: CPK normalized and

the patient's muscular strength and weight were restored. In August 1994 he was rehospitalizated for a colecystectomy. During this period he fasted again and was on a parenteral nutrition for 14, days riboblavin therapy was suspended.

Forty days later, the patient's easy fatiguability returned and his condition progressively worsened until March 1995. Riboflavin supplementation was administered and his symptoms disappeared in 15 days.

Morphological study

Muscle biopsy was frozen and processed for routine histochemistry.

Biochemical studies

Muscle, plasma and urine carnitine fractions are assayed according to a method reported elsewhere [1]. NADH-dehydrogenases (complex I), succinate dehydrogenase (complex II), succinate -cytochrome c reductase (complex II-III), NADH-cytochrome c reductase (complex I-III) and cytochrome c oxidase (COX), (complex IV) were measured in muscle homogenates as previously described [20]. The enzymatic activities of respiratory chain complexes were tested twice on frozen muscle and normalized to citrate synthetase.

In fresh muscle homogenates the following were assayed: ($^{14}CO_2$) production from (1-^{14}C) pyruvate, (1-^{14}C) butyrrate, (1-^{14}C) octanoate, (1-^{14}C) palmitate with and without 2 mM carnitine [8].

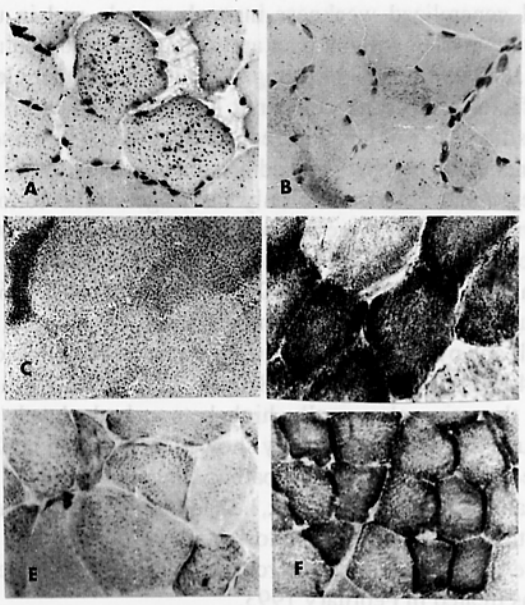

Figure 1. The biopsy of the patient before treatment shows a myriad of lipid-filled vacuoles and mitochondrial subsarcolemmal rims. Oil Red O stain in patient (A) and control muscle (B) (X 40). Succinate dehydrogenase stain in patient (C) and control muscle (D) (X40). COX stain in patient (E) control muscle (F) (X 40).

HPLC method

A muscle specimen was homogenated in buffer and treated as previously described [4]. Each sample was processed in parallel with the same to whom appropriate standards of the three flavins were added for the recovery's evaluation. The recovery ranged from 50 to 95%. Riboflavin (Rb), FMN and FAD were tested in muscle homogenate by high-performance liquid chromatography (HPLC) as described by Batey [4]. With this specific and sensitive method these metabolites were separated by reversed-phase column chromatography (Ultasphere ODS 25 cm Beckman, USA) and quantitated by their native fluorescence at 450 nm (excitation) and 520 nm (emission). using a fluorimetric apparatus (RF-535 Shimadzu, Japan). Calibration was performed using peak area (integrator model C-R3A Schimadzu, Japan). Standard metabolites were purchased from Sigma (St Louis, USA). Standard curves were linear for the three flavines, from 2.5 picomoles to 100 picomoles (Fig. 2).

*Table I. Carnitine and acyl-carnitine levels in the patient's muscle.**

	PATIENT	CONTROL RANGE (N° 32)
TOTAL	4.33	10.5 - 29.5
FREE	1.80	8.3 - 24.3
ACYL	2.54	2.05 - 8.66
RATIO ACYL/FREE	1.41	0.09 - 0.78

* Values are expressed as nmol/mg non collagen protein

Table II. Oxidation of labeled substrates in fresh muscle supernatant.

		PATIENT	CONTROL
[1-^{14}C] PYRUVATE	-	40	75.7 ± 22.6 (8)*
	+	52	126.9 ± 32.2 (8)
[1-^{14}C] BUTYRRATE	-	550	936.6 ± 277.5 (6)♦
	+	455	706.2 ± 339.5 (10)
[1-^{14}C] OCTANOATE	-	111	803.9 ± 412.6 (7)♦
	+	53	1050.4 ± 744.8 (7)
[1-^{14}C] PALMITATE	-	4200	4334.3 ± 2817.7 (14)♦
	+	8236	8321.0 ± 5396.2 (14)

Values are expressed as: (♦) pmol/mg protein/hour; (*) n mol/mg protein/hour mean ± SD
(-) without carnitine
(+) with 2 mM L - carnitine

*Table III. Respiratory chain enzyme activities in patient's muscle.**

		CONTROLS (N° 14)	
	PATIENT	MEAN ± SD	NORMAL RANGE
COMPLEX I	89.5	215.4 ± 42.0	122.5 - 279.4
COMPLEX II	0.8	5.0 ± 1.8	2.0 - 9.2
COMPLEX I-III	8.0	18.3 ± 7.6	6.4 - 35.0
COMPLEX II-III	1.3	4.9 ± 1.9	2.1 - 9.4
COMPLEX IV	6.2	15.5 ± 3.5	9.1 - 21.0
CITRATE SYNTHETASE	472.0	240.0 ± 37.0	189.7 - 330.0

* Values are expressed as nmol/min/mg protein, normalized to citrate synthetase activity.

Results

The muscle biopsy from our patient was studied before treatment with riboflavin. A marked lipid storage was observed in type I fibers with ORO stain (Fig. 1A), succinate dehydrogenase stain showed a faint diffuse reactivity (fig. 1C) and numerous fibers were cytochrome C oxidase negative (Fig. 1E). The patient's skeletal muscle carnitine values were low: free carnitine was 10%, total carnitine was 20% of control, whereas esterified/free carnitine ratio was five-fold normal values (Table I). In muscle homogenate the activity of succinate dehydrogenase was 17% and COX 45% that of normal (Table III). The activity of complex I was reduced to 42% that of controls. All percentages for activities were calculated as a ratio to citrate synthase. In the patient's muscle this enzyme was 200% that of control (Table III).

Beta-oxidation was measured in fresh muscle homogenate using as labeled substrates (1-^{14}C) pyruvate, (1-^{14}C) butyrrate, (1-^{14}C) octanoate and (1-^{14}C) palmitate in the presence or absence of 2 mML-carnitine. The results are shown in Table II. A normal palmitate oxidation was found whereas octanoate oxidation was reduced to 14% and 5% of controls without and with carnitine respectively. Pyruvate and butyrrate oxidations were 50% of normal.

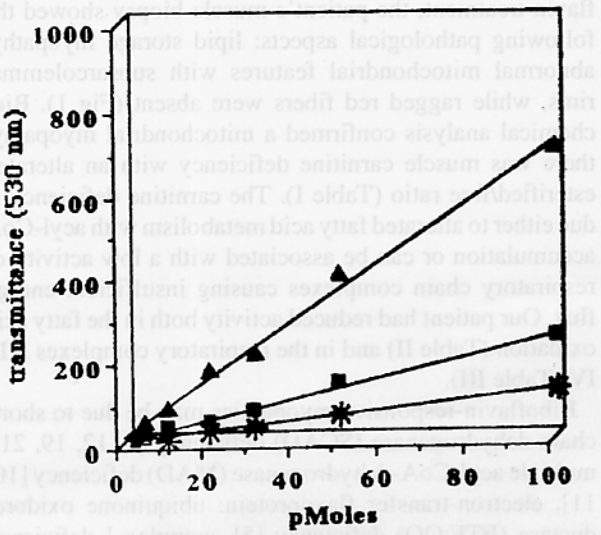

Figure 2. Standard curves for (s) FMN, (n) Riboflavin, (*) FAD are straight and calculated to be respectively: y = 9.9+6.98x R = 1; y = 1.8 + 2.4x R = 1; y = 0.99 + 1.1x R = 1.

To investigate a defect in riboflavin metabolism in patient's muscle we measured riboflavin, FMN and FAD in a frozen muscle sample and compared findings with those made in fresh and frozen control muscle. These compounds are usually assayed only in blood or urine, and no muscle levels are available in the literature. Using a sensitive and specific HPLC method [4] we detect the concentration of these three flavins down to 2.5 picomoles. Standard curves for riboflavin, FMN, FAD are illustrated in figure 2.

The muscle content of riboflavin, FMN and FAD in our patient was similar to control (Table IV).

Discussion

We here have reported findings from morphological and biochemical investigations in the muscle biopsy of a 36 -year- old patient with severe muscle weakness, which improved after riboflavin supplementation. Before ribo-

*Table IV. FAD-FMN riboflavin in human muscle.**

	FAD	FMN	RIBOFLAVIN
PATIENT	21-07	2.20	N.D.
CONTROL FRESH MUSCLE (N° 4)	19.98 ± 4.46	1.96 ± 0.27	N.D.
CONTROL FROZEN MUSCLE (N° 4)	17.90 ± 3.92	1.87 ± 0.34	N.D.

N.D. Non detectable.

* Values are expressed as pmol/mg protein. Mean ± SD

flavin treatment, the patient's muscle biopsy showed the following pathological aspects: lipid storage myopathy, abnormal mitochondrial features with subsarcolemmal rims, while ragged red fibers were absent (Fig 1). Biochemical analysis confirmed a mitochondrial myopathy: there was muscle carnitine deficiency with an alterated esterified/free ratio (Table I). The carnitine deficiency is due either to alterated fatty acid metabolism with acyl-CoA accumulation or can be associated with a low activity of respiratory chain complexes causing insufficient energy flux. Our patient had reduced activity both in the fatty acid oxidation (Table II) and in the respiratory complexes I, II, IV (Table III).

Riboflavin-responsive myopathies may be due to short-chain dehydrogenase (SCAD) deficiency [7, 12, 19, 21], multiple acyl-CoA-dehydrogenase (MAD) deficiency [10-11], electron-transfer flavoprotein: ubiquinone oxidoreductase (ETF:QO) deficiency [5], complex I deficiency [18], combined complex I and II and MAD deficiency [2] and pyruvate dehydrogenase deficiency [17].

Ross [16] indicated a characteristic response of higher organisms to riboflavin deprivation status. In riboflavin deficiency the metabolic pathways of the greatest importance are spared, while non essential pathways may be deranged. We can therefore explain the low oxidation of butyrrate and octanoate by muscle, even though we could not check the organic acid profile in urine while the patient was fasting during hospitalization. Usually SCAD or mild MAD deficiency are recognized from ethymalonic-glutaric aciduria. Complex I and II contain flavin coenzymes and may be involved in riboflavin-responsive myopathy as observed by Antoniozzi et al. [2], although the mechanism underlying this phenomena is still unknown.

Elsewhere, the low activity of complex IV of the mitochondrial respiratory chain has also been reported in association with acyl-CoA-dehydrogenase deficiency [6, 13, 14]. Further investigations are required to clarify the relationship between respiratory chain complexes and of β-oxidation defects.

The normal FAD and FMN contents found in our patient's muscle rule out the following defects in riboflavin metabolism: muscle Rb transport and its transformation to FMN and FAD by flavokinase and FAD-pyrophosphorylase. In our patient, another two primary defects of riboflavin metabolism may play a role: FAD and/or FMN transport in mitochondria and their assembly to apoenzymes may be abnormal [15].

We conclude that in some cases of metabolic myopathy riboflavin treatment may be useful in restoring a normal clinical and biochemical status. More studies are required to indicate the primary defect of Rb metabolism that is involved in such cases, such as cell cultures to investigate the mitochondrial transport of flavins.

Acknowledgments

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