Diagnosis and Therapy of Myophosphorylase Deficiency: Experience with a Group of Italian Patients

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Abstract

Myophosphorylase deficiency (McArdle's disease) is the most frequently diagnosed glycogenosis of muscle. The molecular characterization of the defect at the protein, RNA, and DNA level, while adding to the understanding of the disease, underlies its heterogeneity. We present 18 Italian patients with McArdle's disease, review their clinical, biochemical, and molecular data, and present the results of a small therapeutic trial with propionyl-L-carnitine. In our patients there was a prevalence of mild clinical presentation, which did not correlate with a distinctive biochemical or molecular pattern. The distribution of genomic mutations reflected the particular ethnic background of the Italian population, and it was unlike other reported studies.

Patients with McArdle's disease demonstrate decreased O_2 utilization and metabolic changes suggestive of impaired oxidative metabolism. Propionyl-L-carnitine treatment causes some changes suggestive of better activation of lipid metabolism.

Key words: glycogen phosphorylase, McArdle's disease, glycogenosis type V, L-Carnitine, propionyl-L-carnitine.

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Myophosphorylase (M-GP) deficiency since its original description by McArdle [16] constituted a methodological model for the clinical, biochemical, and more recently molecular approach to muscle metabolic dysfunction in man.

The lack of lactate production by exercising muscle observed by McArdle in 1951 was the key which led to the hypothesis that a defect in glycogen breakdown was the cause of the disorder. However it took 8 years to prove the biochemical defect [20, 25], 12 more years to sort out between patients without any protein and patients with inactive but immunologically detectable enzyme [8], 16 more years to show the remarkable heterogeneity at the transcript level [9, 17, 26], and only in the last two years a genomic mutation was associated conclusively with the disease [29].

During these 40 years of McArdle's disease history several works focused on the pathophysiology of the disease. As a consequence the apparently simple concept of ATP shortage due to impaired glycolysis was substituted by the complex picture of unbalanced metabolic pathways leading to muscle energy failure [14]. In the same way the initial therapeutic strategy of by-passing the glycogen unavailability in muscle by rising blood glucose, changed to a more comprehensive approach which favours the compensatory potential of alternative metabolic pathways.

In spite of this long path in studying and characterizing the disease, several questions about etiology, pathophysiology, and treatment, remain open.

In this work we present our experience with a large group of Italian patients in light of the new advances of molecular genetics, and present the results of a preliminary open therapeutic trial with propionyl-carnitine. We choose propionyl-carnitine as candidate therapy because it could act at three levels: 1) on the Krebs cycle, with an anaplerotic effect thanks to its propionyc moiety [5], 2) on fatty acids oxidation easing their uptake into mitochondria thanks to carnitine [12], 3) on ammonia clearance functioning as glutamate precursor [34].

Materials and Methods

Patients

18 patients with biopsy proven myophosphorylase deficiency currently followed in the Centres of Padova (13 patients), Pisa (2 patients), and Messina (3 patients). The essential data on the patients are summarized in table 1.

Biochemistry

Glycogen phosphorylase (GP) activity and glycogen content were assayed in muscle homogenate as described [6]. Protein studies were carried out by SDS-PAGE with a linear gradient of 8-13% in the separating gel. The slabs were either stained with coomassie-blue or blotted onto nitrocellulose and immunostained with specific anti M-GP antiserum as described elsewhere [19].

Molecular genetics

Total RNA purified from muscle specimens by the RNA-zol kit (Cinna-Biotex Labs, Houston, TX) was studied by Northern blot or by PCR amplification after reverse transcription (RT) as described elsewhere [19]. cDNA obtained by RT or genomic DNA extracted from muscle or blood cells were PCR amplified using specific primers covering the entire coding sequence of the M-GP gene [29, 30]. The presence of the described mutations (codons 49, 204, 291, 396, 542, 654, 708) was tested by restriction analysis (respectively with NlaIII, HaeIII, KspI, BglI, MaeII, MscI) of amplified fragments and resolution on 3% agarose gel (2% Nu-Sieve 1% agarose) or by direct sequencing (codon 708).

Exercise studies

Four hours after a light and balanced meal 5 patients and 5 controls completed a progressive exercise test on bicycle ergometer (Ergomed 740, Siemens). Work load was increased stepwise by 20 W every 3 min until exhaustion (inability of the subject to sustain the constant pedalling on the bicycle ergometer). After completion of the exercise test the subjects lay down for 30 min. Before, during and after exercise, the following parameters were measured: breath rate, ventilation (Ve/min) (Biomed inc. spirometer), O_2 uptake (VO_2), CO_2 production (VCO_2) (Datex O_2/CO_2 analyzer), blood pressure (measured with a sphygmomanometer on the left arm every 2 min), PO_2, PCO_2, and pH on arterialized blood (every 3 min), blood glucose, lactate, pyruvate, ammonia, free fatty acids (FFA), K+, Pi, creatin kinase (CK) (on venous blood drawn from the right antecubital vein every 3 min during effort and after 5 and 30 min during recovery). Plasma carnitine and its fractions were measured at rest, at exhaustion, 5 and 30 min during recovery. PO_2, PCO_2, and pH were measured on a ABL-30 Radiometer (Copenhagen), lactate and pyruvate and CK were measured spectrophotometrically, FFA, glucose, ammonia, Pi, and K+ were assayed by routine methods. For plasma carnitine and its fractions we used the radiochemical assay as modified by Parvin and Pande [21]. A three channel EKG was recorded during the entire test.

Propionyl-carnitine trial

The same protocol was repeated in the five patients under acute propionyl-carnitine treatment. The treatment consisted in a first i.v. infusion (25 mg/Kg) 24 hours before the test and a second i.v. infusion just before (15 mg/Kg) and during (15 mg/Kg) the effort. The two exercise tests were separated by at least 15 days. The results of the first exercise test were compared for each patient with the performance of the second test.

Table 1. Essential data of McArdle's patients.

		n. of patients	%
Sex	M	12	67
	F	6	33
Age at diagnosis	< 30	7	39
	30-50	7	39
	> 50	4	18
Age of Onset	< 10	10	56
	10-35	2	11
	> 35	6	33
Family	sibs	5	28
	+	3	16
	-	10	56

Results

Clinical presentation

Among our patients males outnumbered females (2:1). Age of onset was below 10 years in 56%, late onset (over 35 year) was observed in 33%. Family history was positive for 44% (table 1). Of particular interest was a family to which belonged 3 of our sibling cases. In this pedigree there was no reported parental consanguinity, and 3 out of 4 siblings were affected.

The sign which led the patients to the neurologist and which addressed the diagnostic work-out was myoglobinuria in all the cases in which it was reported (39%). In the other patients the key element for diagnostic orientation was elevated blood CK, although the complaint which took the patients to the doctor was weakness or exercise intolerance. The average time between the probable age of onset and the diagnosis was 16 ± 9 years (range 1-29 years). In most patients the clinical picture did not change between pre- and post-diagnosis years, with the exception of the episodes of myoglobinuria which became much less frequent after the correct diagnosis was established. None of the patients who experienced myoglobinuria developed renal failure. In 3 patients there was a progressive deterioration with fixed weakness, hypotrophy, and severe exercise intolerance. Cardiovascular (hypertension or

cardiomyopathy) or cerebrovascular (ischemic stroke) involvement was observed in 4 patients. Ischemic lactate test and EMG were performed in all patients but one, in whom the diagnosis was suspected on the ground of family history, and confirmed by molecular testing on genomic DNA. Lactate test was always positive, while the EMG showed abnormalities suggestive of myopathy in only 4 cases (table 2).

Most patients (13/16) do not follow a specific therapy, and report no significant limitations in their daily activities once they avoid strenuous exercise. One patient uses oral fructose supplementation occasionally when planning unusual efforts, one patient is currently assuming oral propionyl-carnitine (500 mg t.i.d.) and reports subjective benefit and no side effect, one is following a high protein diet (28% proteins) with limited benefit.

Biochemical and molecular characterization

A summary of biopsy findings, protein and transcript analysis is provided in table 3.

Muscle biopsy was the diagnostic procedure taken in all cases after the ischemic lactate test. PAS positive subsarcolemmal blebs were observed in 13 biopsies, while in 3 biopsies the PAS + staining was diffusely increased without vacuolization. Histoenzymatic stain for GP was completely negative in 14 cases, while in 3 cases scattered regenerating GP-positive fibres were observed. GP activity was undetectable in 12 cases (70%), and below 3% of control in 4 (22%). Only in one case the activity was 13% of control. Glycogen content was elevated in all 13 cases tested. Immunodetectable M-GP protein was absent in 14 cases and present in reduced amount in 1.

M-GP mRNA was assayed in 15 patients. It was detected by RT-PCR in 11, and was absent in 4.

The profile of the genomic mutations detected in this group of patients is provided in table 4.

Only 2 patients were homozygous for the R49X mutation. No mutation was detected in 5 patients. Four patients were compound heterozygous for R49X and a missense mutation: L396P in 3 siblings, E654R in a sporadic case. In six patients a mutation could be demonstrated only in one allele: in two siblings it was the missense mutation L396P, in four sporadic cases it was the R49X nonsense mutation.

Exercise test

In all patients exhaustion was reached much earlier and at lower workload (37.4 ± 9.6% of expected) than control. VO_2 max was significantly reduced (56.6 ± 19.37%) even comparing the VO_2 of controls exercising at the same workload at which the patients exhausted. Ventilation was comparable to controls. Respiratory ratio (R) was below 1 in all patients (0.77 ± 0.11). Cardiovascular response to exercise (heart rate, blood pressure) was not different between patients and controls.

The biochemical changes observed in controls during and after exercise were: blood pH decrease, rise in blood lactate and pyruvate (280% and 170%), slight rise in glucose (115%), persistent slight rise in FFA (110%), moderate increase of ammonia (150%) (fig. 1), no changes in CK, Pi, K+.

In the patients there was no change in blood pH and lactate. Pyruvate showed a significant decrease during exercise (-25 ± 3%, $p < 0.02$), and ammonia increased more then fourfold ($p < 0.001$). FFA declined during effort and rebounded to higher than control during recovery (fig. 1). There were no changes in Pi, K+, and CK, which was

Table 2. Clinical and strumental data in McArdle's patients.

Simptoms and signs	n. of patients	%
Exercise intolerance	18	100
Myoglobinuria	7	39
Weakness	6	33
Hypotrophy	4	22
High CK	17	94
Myopathic EMG	4	22

Table 3. Biopsy findings in McArdle's patients.

Biopsy finding		n. of patients	%
Increased Glycogen		16	100
GP Activity	0	12	70
	< 3%	4	23
	> 3%	1	7
GP Protein (Western Blot)	Absent	14	94
	Present	1	6
GP mRNA (RT-PCR)	Absent	4	27
	Present	11	73

Table 4. Genotype of McArdle's patients.

Genotype	n. of patients	%
49/49	2	11
49/-	4	22
49/654	1	6
49/396	3	17
-/396	2	11
-/-	6	33

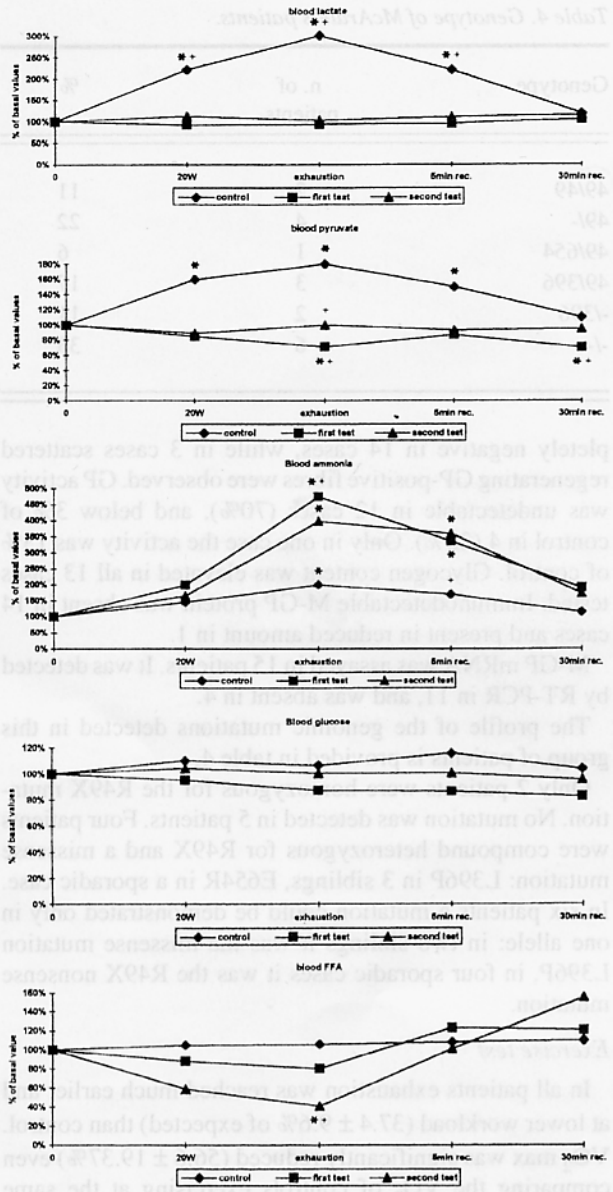

*Figure 1. Variations in blood metabolites during and after exercise test in controls (u), patients (l), and patients under propionyl-carnitine treatment (s). Values are expressed as % of baseline levels. * p < 0.05 compared to baseline. + p < 0.05 compared to other groups at the same time point.*

elevated in patients at the baseline but did not rise any further during or after exercise.

Plasma free carnitine concentration did not change in patients and control after effort. In controls there was a significant increase in long-chain acyl carnitine (166% of baseline values, $p < 0.05$) which was not observed in patients. Medium-chain acyl-carnitine was moderately increased during recovery in both patients and controls (fig. 2).

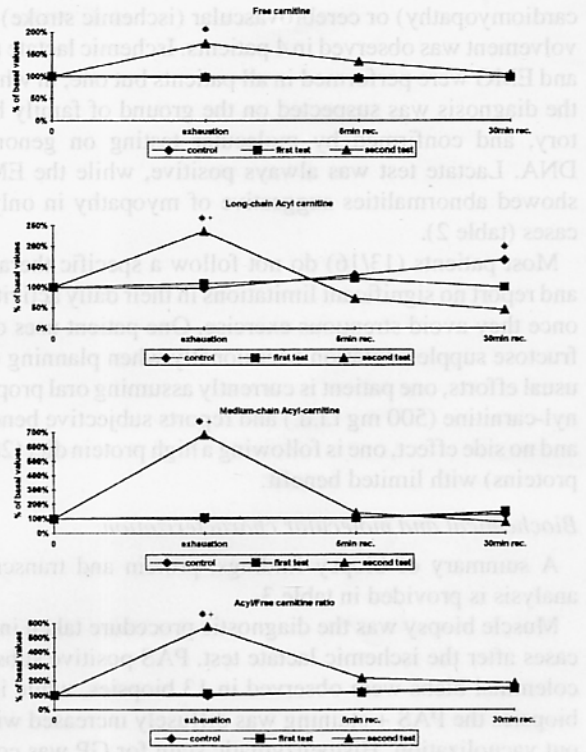

*Figure 2. Carnitine and its fractions during and after exercise test in controls (u), patients (n), and patients under propionyl-carnitine treatment (s). Values are expressed as % of baseline levels. * p < 0.05 compared to baseline. + p < 0.05 compared to other groups at the same time point.*

Propionyl-carnitine trial

Maximal workload, ventilation, VO_2 max, heart rate, and R did not differ significantly between pre and post-treatment exercise test. There was a trend towards lower ammonia and more stable pyruvate levels in the post-treatment test which was significant ($p < 0.05$) only for pyruvate (fig. 1). There was a significantly ($p < 0.05$) more pronounced decrease in FFA after effort in the post-treatment test (-60% vs -19%) (fig. 1).

No difference was observed in glucose, CK, K+, Pi, lactate between the two tests.

The treatment with propionyl-carnitine caused a significant increase of baseline plasma free and acyl-carnitine ($p < 0.001$). The increase was maximal for the medium-chain (22 fold) and long-chain fraction (366%), and minimal for free carnitine (150%). At exhaustion there was a significant further increase of all carnitine fractions, which returned to baseline during recovery. Again the widest variation (685%) was observed for medium-chain acyl carnitine (fig. 2).

Discussion

In the last 20 years myophosphorylase deficiency was the glycolysis defect most frequently diagnosed in our

Centres. 50% of patients presented clinically with a very mild picture of exercise intolerance and no myoglobinuria, 20% of patients presented with a more severe picture of fixed weakness, muscle wasting, limitation in daily living activities, and the others had the classical picture of episodic myoglobinuria (table 2). This distribution is slightly different from what has been reported in other works [7, 11, 18] in which there was a larger proportion of patients with moderate (50%) and severe (25%) presentation. The diagnosis was suspected in 3 of 5 of our sibling cases on the ground of familiarity, and only under specific questioning the patients recalled their poor performance in heavy exercise, to which they had learned to adapt, to the point to consider it "normal". An other relevant difference between ours and previously reported series is the higher proportion of late onset cases (33% vs 6-8%), and almost all the patients with late onset presented a very mild phenotype. The shift towards very mild presentation in our group could be due to these factors.

The most sensitive non-invasive diagnostic test was the ischemic lactate test, while the abnormality which most frequently started the specific diagnostic procedure was elevated serum CK. In our experience a compatible clinical history and a positive ischemic lactate test are sufficient to propose the patient for a muscle biopsy. A possible exception is a patient belonging to a family in which M-GP deficiency has been diagnosed and in which a relevant mutation has been demonstrated in both alleles. In this patient the diagnosis can be conclusively reached by analysis of blood genomic DNA.

The protein and mRNA study of our patients showed results similar to other reported series (fig. 3a and 3b).

The presence of immunodetectable inactive M-GP protein is observed in less than 10% of patients, and M-GP mRNA can be detected in more than 70% of patients. The presence of immunodetectable protein was demonstrated in only one of our patients, who was compound heterozygous for the nonsense R49X and the missense E654K mutations. His genotype is compatible with the reduced translation of an inactive protein as observed. However the same is not observed when R49X is found together with other missense mutations (L396P and G204S), albeit in these cases a reduced amount of normal size transcript is usually found. No transcript is usually detectable in patients homozygous for the R49X mutation (this work, 2, 29). It seems therefore that the molecular consequence of the R49X mutation is a reduction in stable transcript, which is maximal when the mutation is present on both alleles. The presence on the other allele of a missense mutation seems to lead to translation of a full size inactive protein only when the mutation is located close to the COOH terminus as in the case of the E654K mutation. Missense mutations located upstream usually are not associated with stable translation products, possibly because of rapid protein breakdown and degradation.

The most frequently observed mutation in our patients was the R49X (12 alleles), followed by the L396P (5

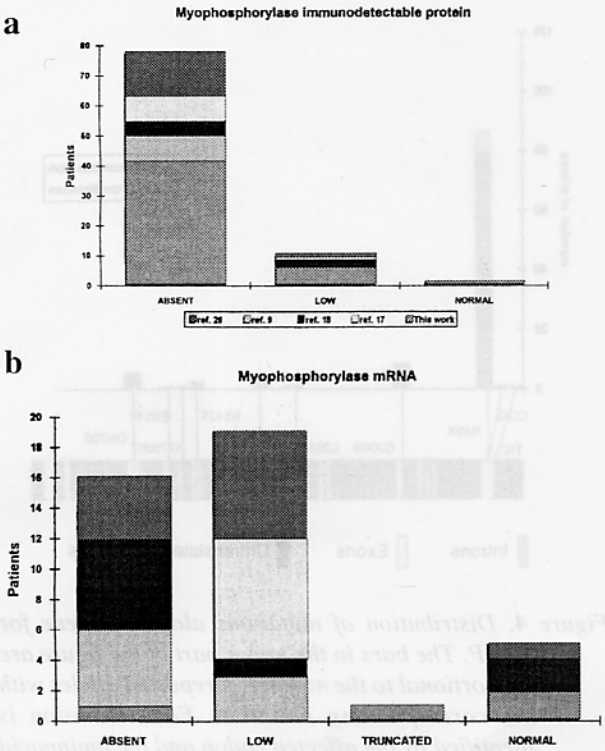

Figure 3. Molecular characterization of myophosphorylase deficiency patients at the protein (3a) and at the transcript (3b) level in this work and in published studies.

alleles), while other mutations (G204S, K542T, D708) reported with some frequency in other studies [3, 29, 30, 31] were not found. There is a significant ($p < 0.05$) lower proportion of alleles with the R49X mutation in our group of patients (33%) compared with those reported by others (60%) [3, 29-33] (fig. 4). These data are consistent with a different genetic background between the Italian patients presented in this study and the patients from the UK and the USA. A different distribution of the mutations associated with M-GP deficiency in different populations is confirmed by the presence in certain ethnic groups (Japanese, patients from Northeastern Italy) of "private" mutations such as the deletion of codon 708 described thus far only in Japanese patients [30, 31] and the L396P mutation described almost exclusively in patients from the Veneto region of Italy [33].

Considering the clinical picture and the biochemical and molecular data, we did not observe any correlation which could explain the clinical heterogeneity. In particular we could not group together patients with less pronounced glycogen muscle storage, residual GP activity in their muscle biopsy, with immunodetectable M-GP protein, or with a particular mutation. Similar results have been obtained by others [7, 28]. Factors other than genotype and biochemical phenotype must influence the clinical presentation of the disease. Among the possible elements are

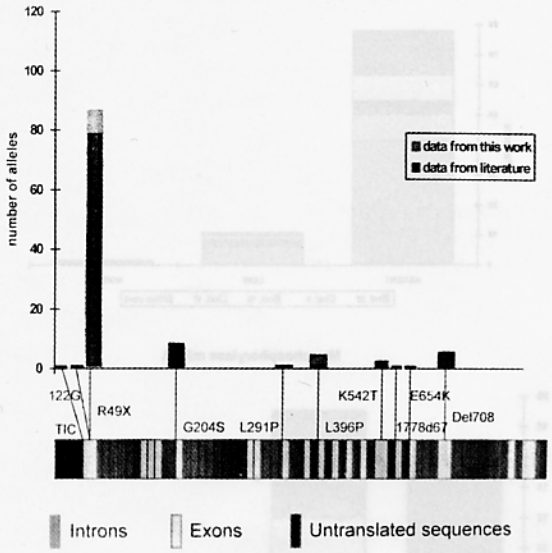

Figure 4. Distribution of mutations along the gene for M-GP. The bars in the upper part of the figure are proportional to the number of reported alleles with the corresponding mutation. Each mutation is identified by the affected codon and the aminoacid changes which causes. Exceptions are: X: stop codon; 122G: insertion of TT in place of the G in position 122; TIC: mutation in the translation initiation codon; Del708: deletion of the codon 708.

hormonal habit, diet, exercise pattern, efficiency of alternate energy-providing pathways.

The results of the exercise test in our small group of patients indicate that the ability to utilize O_2 during exercise is significantly impaired in M-GP deficient patients, as was already suggested by previous studies [10]. During maximal effort all readily available oxidative substrata are utilized as suggested by the decrease in glucose and pyruvate levels. The activation of lipid oxidation occurs with delay and seems desynchronized with the effort as suggested by the behaviour of FFA and acyl-carnitine.

The disproportionate increase in ammonia observed in McArdle's patients suggests that energy is initially provided to exercising muscle by ATP resynthesis via ADP condensation by myokinase and consequent increase in AMP deamination [14], or by deamination of aminoacids [4, 35]. We did not observe the changes in Pi and K+ reported by others [1, 22] probably because venous blood does not reflect closely enough (as NMR spectroscopy or arterial blood) the acute changes occurring in muscle during exercise.

The treatment with propionyl-carnitine was only marginally effective. The significant changes were the increase at exhaustion and the subsequent decrease during recovery of FFA and acyl-carnitine, paralleled by the more stable level of pyruvate at exhaustion and recovery. These data suggest a more efficient activation of lipid metabolism with relative sparing of pyruvate, and are consistent with the rationale of the therapy with propionyl-carnitine. The effect on plasma ammonia reported by others with propionyl-carnitine [34] was only partially reproduced in our study, but in both works the changes were not significant.

These results indicate a potential benefit from the use of propionyl-carnitine in patients with McArdle's disease, which could be used in a more prolonged trial with oral formulation.

Other therapeutic strategies reported thus far for McArdle's disease include glucose, fructose or ribose infusion or supplementation [1, 14, 36], glucagon, norepinephrine or heparin infusion [15, 23, 24], high protein diet [13, 27]. Among our patients only a few felt the need of some therapeutic intervention, since in most cases the patient was able to avoid episodes of myoglobinuria, muscle cramps, and myalgias simply by restricting his physical activity. In the patients requiring therapy we found that high protein diet, oral propionyl-carnitine, and occasional use of oral fructose were the most easily accepted therapies. An attempt to treat a severely affected patient with subcutaneous norepinephrine infusion was unsuccessful and associated with cardiovascular side effects.

In conclusion this study demonstrates the clinical, biochemical and molecular heterogeneity of McArdle's disease, indicates that the various genomic mutations associated with the disease are distributed differently in different populations, confirms the involvement of oxidative metabolism in the energy failure affecting M-GP deficient muscle, suggests that propionyl-carnitine could offer some advantages in the therapy of the disease, and deserves further trials.

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References

[1] Argov Z, Bank WJ, Maris J, Chance B: Muscle energy metabolism in McArdle'syndrome by in-vivo phosphorus magnetic resonance spectroscopy (^{31}p MRS). *Neurology* 1987; 37: 1720-1724.

[2] Bartram C, Edwards RHT, Clague J, Beynon RJ: McArdle's disease: a nonsense mutation in exon 1 of the muscle phosphorylase gene explains some but not all cases. *Hum Mol Genet* 1993; 2: 1291-1293.

[3] Bartram C, Beynon RJ, Edwards RHT: Mutational analysis of McArdle's disease. *Muscle & Nerve* 1994, (suppl. 1): S125.

[4] Coakley JH, Wagenmakers AJM, Edwards RHT: Relationship between ammonia, heart rate, and exertion in McArdle's disease. *Am J Physiol* 1992; 262: E167-E172.

[5] Davis EJ, Spydevold O, Bremer J: Pyruvate carboxylase and propionyl-CoA carboxylase as anaplerotic enzymes in skeletal muscle mitochondria. *Eur J Biochem* 1980; 110: 255-262.

[6] DiMauro S, Hartwig GT, Hays AP, Eastwood AB, Franco R, Olarte M, Chang M, Roses AD, Fetell M, Schoenfeldt RS, Stern LZ: Debrancher deficiency: neuromuscular disorders in five adults. *Ann Neurol* 1979; 5: 422-436.

[7] Di Mauro S, Tsujino S: Nonlysosomal glycogenoses, in Engel AG and Banker BQ (eds): *Myology, 2nd edition*. New York, McGraw-Hill, 1994, pp 1554-1576.

[8] Dreyfus JC, Alexandre Y: Immunological studies on glycogen storage disease type III and V. Demonstration of the presence of an immunoreactive protein in the case of muscle phosphorylase deficiency. *Biochem Biophys Res Commun* 1971; 44: 1364-1370.

[9] Gautron S, Daegelen D, Mennecier F, Dubocq D, Khan A, Dreyfus JC: Molecular mechanisms of McArdle's disease (muscle glycogen phosphorylase deficiency). RNA and DNA analysis. *J Clin Invest* 1987; 79: 275-281.

[10] Haller RG, Lewis SF, Cook JD, Blomqvist CG: Myophosphorylase deficiency impairs muscle oxidative metabolism. *Ann Neurol* 1985; 69: 1231-1235.

[11] Hers HG, Van Hoof F, Barsy T: Glycogen storage diseases, in Scriver CR, Beaudet AL, Sly WS, Valle D (eds): *The metabolic basis of inherited disease*. New York, McGrow Hill, 1989, pp 425-452.

[12] Hoppel C: The physiological role of carnitine, in Ferrari R, DiMauro S, Sherwood G (eds): *L-carnitine and its role in medicine: from function to therapy*. London, Academic Press, 1992, pp 5-19.

[13] Jensen KE, Jakobsen J, Thomsen C, Heriksen O: Improved energy kinetics following high protein diet in McArdle's syndrome. A ^{31}P magnetic resonance spectroscopy study. *Acta Neurol Scand* 1990; 81: 499-503.

[14] Lewis SF, Haller RG: The pathophysiology of McArdle disease: clues to regulation in exercise and fatigue. *J Appl Physiol* 1986; 61: 391-401.

[15] Kono N, Mineo S, Shimizu T, Kang J, Nonaka K, Tarui S: Metabolic basis of improved exercise tolerance: muscle phosphorylase deficiency after glucagon administration. *Neurology* 1984, 34: 1471-1476.

[16] Mc Ardle B: Myopathy due to defect in muscle glycogen breakdown. *Clin Sci* 1951; 10: 13-33.

[17] Mc Conchie SM, Coacley J, Edwards RHT, Beynon RJ: Molecular heterogeneity in McArdle's disease. *Biochim Biophys Acta* 1991; 1096: 26-32.

[18] Mantle P, Lauffart B, Atack J, Lane RJM: Absence of biochemical heterogeneity in McArdle's disease. *J Neurol Sci* 1987; 78: 63-70.

[19] Martinuzzi A, Vergani L, Carrozzo R, Fanin M, Bartoloni L, Angelini C, Askanas V, Engel WK: Expression of muscle-type phosphorylase in innervated and aneural cultured muscle of patients with myophosphorylase deficiency. *J Clin Invest* 1993; 92: 1774-1780.

[20] Mommaerts WFHM, Illingworth B, Pearson CM, Guillory RJ, Seraydarian K: A functional disorder of muscle associated with the absence of phosphorylase. *Proc Natl Acad Sci USA* 1959; 45: 781-797.

[21] Parvin R, Pande SV: Microdetermination of (-) carnitine acetyl-transferase activity. *Anal Biochem* 1977; 79: 190-201.

[22] Paterson DJ, Friedland JS, Bascom DA, Clement ID, Cunningham DA, Painter R, Robbins PA: Changes in arterial K+ and ventilation during exercise in normal subjects and subjects with McArdle's syndrome. *J Pysiol (London)* 1990; 429: 339-348.

[23] Porte DJr, Crawford DW, Jennings DB, Aber C, McIlroy MB: Cardiovascular and metabolic responses to exercise in a patient with McArdle's syndrome. *New Engl J Med* 1966; 275: 406-412.

[24] Rowland LP, Fahn S, Schotland DL: McArdle's disease. *Arch Neurol* 1963; 9: 325-342.

[25] Schmid R, Robbins PW, Traut RR: Glycogen synthesis in muscle lacking phosphorylase. *Proc Natl Acad Sci USA* 1959; 45: 1236-1240.

[26] Servidei S, Shanske S, Zeviani M, Lebo R, Fletterick R, DiMauro S: McArdle's disease: biochemical and molecular genetic studies. *Ann Neurol* 1988; 24: 774-781.

[27] Slonim AF, Goans PJ: Myopathy in McArdle's syndrome. Improvement with high protein diet. *New Engl J Med* 1985; 312: 355-359.

[28] Toescu V, Bartram C, Phoenix J, Beynon RJ, Edwards RHT: Correlation of clinical, histopathological and genetic data in McArdle's disease. *Muscle & Nerve* 1994 (Suppl. 1); S125.

[29] Tsujino S, Shanske S, DiMauro D: Molecular genetic heterogeneity of myophosphorylase deficiency (McArdle's disease). *New Engl J Med* 1993; 329: 241-245.

[30] Tsujino S, Shanske S, Nonaka I, Eto Y, Mendell JR, Fenichel GM, DiMauro S: Three new mutations in patients with myophosphorylase deficiency (McArdle's disease). *Am J Hum Genet* 1994; 54: 44-52.

[31] Tsujino S, Shanske S, Goto Y, Nonaka I, DiMauro S: Two mutations, one novel and one frequently observed, in Japanese patients with McArdle's disease. *Hum Molec Genet* 1994; 3: 1005-1006.

[32] Tsujino S, Rubin LA, Shanske S, DiMauro S: An A-to-C substitution involving the translation initiation codon in a patient with myophosphorylase deficiency (McArdle's disease). *Hum Mutat* 1994; 4: 73-75.

[33] Tsujino S, Shanske S, Martinuzzi A, Heiman-Patterson T, DiMauro S: Two novel missense mutations in Caucasian patients with myophosphorylase deficiency (McArdle's disease). *Hum Mutat* 1995; 6: 276-77.

[34] Wagenmakers AJM, Coakley JH, Edwards RHT: Propionyl-L-carnitine reduces plasma ammonia in patients with McArdle's disease, in Benzi G (ed): *Advances in Myochemistry 2*. London, John Libey Eurotext Ltd., 1989, p 195.

[35] Wagenmakers AJ, Coakley JH, Edwards RH: Metabolism of branched-chain amino acids and ammonia during exercise: clues from McArdle's disease. *Int J Sports Med* 1990; 11: S101-S113.

[36] Wagner DR, Zollner N: McArdle's disease: successful symptomatic therapy by high dose oral administration of ribose. *Klin Wochenschr* 1991; 69: 92.