Università degli Studi di Udine

Dipartimento scienze mediche e biologiche

Dottorato di ricerca in Scienze biomediche e biotecnologiche

Ciclo XXVII

Functional evaluation of muscle oxidative metabolism in metabolic myophaties.
A cross-talk between exercise physiology and clinical medicine

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Contents

SUMMARY

Metabolic myopathies are a heterogeneous group of diseases characterized by genetically determined defects which impair skeletal muscle energy production and/or function. These diseases include errors of glycogen metabolism, lipid metabolism, and mitochondrial respiratory chain. The most common clinical features are muscle weakness, pain, easy fatigability, cramping and, sometimes myoglobinuria due to muscle fiber necrosis. Exercise intolerance is another hallmark of clinical features of metabolic myopathies. As a result, patients note undue fatigue and dyspnea during low levels of exertion, including moderate activities of daily living. Symptoms are usually first experienced in childhood or early adulthood, however, late-onset is well recognized.

The diagnostic process of these diseases usually begins with a careful medical history, a physical and neurological examination to assess reflexes, strength and the distribution of weakness. Creatine kinase is an extremely useful laboratory test for the evaluation of patients with a suspected myopathy and electromyography may be used to rule out a number of other neuromuscular disorders that cause similar patterns of weakness. As for exercise testing, the six minute walking test and the forearm ischaemic lactate test have traditionally been employed to screen for suspected disorders of glycogen metabolism; however, they have been superseded by improved biochemical and genetic techniques. Confirmation of the diagnosis usually requires muscle biopsy and/or molecular genetic testing.

Functional evaluation of oxidative metabolism during exercise provides information regarding the physiological responses required by the cardiovascular and respiratory systems to meet the metabolic demands of the skeletal muscle. Moreover, the study of the physiological adjustments to exercise in patients is of extreme interest also from a “basic science” point of view, allowing to investigate aspects related to the regulation and integration of physiological and bioenergetic responses. Although exercise testing is widely utilized by cardiology, pulmonary, and sports medicine clinicians as a means to assess heart failure, respiratory disease, or athletic capacity, only few neurologists utilize a comprehensive assessment of oxidative metabolism to clarify the etiology of exercise intolerance and unexplained dyspnea among patients with metabolic myopathies.

In previous studies our group applied on mitochondrial myopathies (MM) and McArdle’s disease (McA) patients two non−invasive methods of functional evaluation specifically aimed at oxidative metabolism at the skeletal muscle level. The variables of functional evaluation that we investigated were: A) Skeletal muscle oxygenation indices during exercise, obtained by near−infrared spectroscopy (NIRS) and taken as estimates of the capacity of O_2 extraction; B) Kinetics of adjustment of pulmonary O_2 uptake ($V'O_2$ kinetics) during the transition from rest to exercise. We demonstrated that these methods allow to identify and quantify, in MM and in McA, the metabolic

impairment. Moreover, these studies represent a typical example of “translational medicine”, in which methods and tools developed over the years in the exercise physiology laboratory are taken to the bed of the patient.

In this thesis will be reported data of four studies in which the above−mentioned tools of functional evaluation of muscle oxidative metabolism were utilized, with specific purposes, on patients with metabolic myopathies.

In the first study we evaluated, during a 24-month follow-up, cardiovascular and metabolic responses to exercise of a 50-yr-old patient with glycogen storage disease type II (Pompe disease) undergoing enzyme replacement therapy (ERT). At the same constant-workload submaximal exercise, rate of perceived exertion, pulmonary ventilation, and heart rate were lower during ERT versus pre-treatment, suggesting an increased exercise tolerance. Peak oxygen uptake increased by approximately 35% after 1 month of treatment and did not significantly change thereafter. Also, peak cardiac output significantly increased during ERT, whereas peak skeletal muscle fractional O_2 extraction was unchanged compared with pre-treatment. Thus, this case report suggest that ERT may increase peak exercise capacity and exercise tolerance at submaximal workloads in patients with glycogen storage disease type II after 1 month of therapy, without no further changes occurring up to 24 months.

In the second study, we followed the same approach of the case study previously mentioned and we evaluated the effects of 12-month of ERT on physiological variables related to exercise tolerance of four patients with Pompe disease. Patients performed an incremental exercise on a cycle ergometer, up to voluntary exhaustion, before and after 12 months of ERT. Peak workload and oxygen uptake values significantly increased after ERT whereas the observed increases of both peak cardiac output and the NIRS-determined peak skeletal muscle fractional O_2 extraction were not statistically significant. These findings suggest that in glycogen storage disease type II patients enzyme replacement therapy is associated with a mild improvement of exercise tolerance. Since exercise training could improve exercise tolerance, motor function and muscle strength, counteracting the general deconditioning typical of chronic diseases, in the future may be interesting to evaluate if exercise training could be helpful in increasing ERT clinical efficacy, improving patients' muscle function and ameliorating their quality of life. A new study based upon a collaboration between neurologists and exercise physiologists has now started and it should give the opportunity to better investigate crucial issues related to patients’ follow-up and treatment.

In the third study we evaluated in McArdle’s (McA) patients whether a first bout of exercise determines a sudden decrease in heart rate (HR) and an improved exercise tolerance (the so-called “second-wind” phenomenon) during a second bout, separated by the first by a few minutes of

recovery. A second-wind phenomenon (marked decrease in heart rate and in the rating of perceived exertion) was indeed observed in McA patients during the second of two consecutive 6-min constant-work rate submaximal exercises. The second wind was associated with changes of physiological variables, suggesting an enhanced skeletal muscle oxidative metabolism: enhanced O_2 extraction; signs of better matching between intramuscular O_2 delivery and O_2 utilization; disappearance of the "slow component" of pulmonary VO_2 kinetics. The second wind was not described in McA patients after a longer (18-min) recovery period or in patients affected by a mitochondrial myopathy who have similar exercise intolerance. Besides being of interest from a basic science point of view (elucidating the mechanisms responsible for the second wind in McA patients), results of the present study are of interest also from a clinical point of view, since they identify a method (a warm up moderate-intensity exercise, carried out a few minutes before performing a task) capable of significantly increasing exercise tolerance in these patients.

Finally, still unpublished data of another study are presented in this thesis, demonstrating the utility of non−invasive functional evaluation methods utilized by physiologists in the follow−up of patients as well as in the evaluation of the effects of therapies and/or rehabilitation intervention (i.e. exercise training). Since at present the therapeutic interventions available for metabolic myopathies patients are very limited and evidence from the literature suggests that moderate−intensity aerobic exercise training represents a safe intervention, the variables of functional evaluation determined by the above−mentioned tools were utilized to evaluate, in MM and McA patients, the effects of a program of moderate−intensity aerobic exercise training carried out by the patients at their home. Peak O_2 uptake, variable evaluating maximal aerobic power, and peak skeletal muscle (vastus lateralis) fractional O_2 extraction, as estimated by near-infrared spectroscopy (NIRS), increased significantly with training both in MM and in McA. Thus, training induced an increase of exercise tolerance at least in part due to a reduction of the impaired fractional O_2 extraction by skeletal muscles. Moreover, training significantly speeded the $V'O_2$ kinetics, even though only in the patients who had presented, before training, markedly slow $V'O_2$ kinetics (i.e. sign of the most pronounced metabolic impairment). Surprisingly, the improvements in exercise tolerance obtained by the training program did not determine an increase in the habitual level of physical activity evaluated a couple of months after the termination of the training program.

Overall, the results of the studies reported in this thesis demonstrate that, within a translational approach, a combination of traditional and more innovative functional evaluation methods can effectively detect the functional improvements of patients with metabolic myopathies following pharmacological and/or exercise interventions, yielding insights also on the mechanisms of the improvements at the pathophysiological level. Thus, functional evaluation of oxidative metabolism

by non−invasive methods could be usefully employed in the diagnostic process of metabolic myopathies, in the follow−up of patients, and in the evaluation of the effects of therapies and/or rehabilitation interventions. Moreover, the analysis of the physiological and bioenergetics adaptations to exercise in patients with metabolic myopathies represents an interesting model to investigate and elucidate, in vivo, the regulation of basic physiological processes.

DEFINITION OF METABILIC MYOPATHY

Muscle function can be disturbed by exogenous influences impinging on the muscle (e.g., physical trauma, toxic substances, infectious organisms, and endocrine and other systemic disorders), as well as by inherited defects in its structure and biochemistry. Any disorder in which muscle dysfunction arises from some genetic defect in muscle itself or from damage directly to the muscle is termed a *myopathy* ([Mastaglia, FL and Detchant, Lord Walton (eds): Skeletal Muscle Pathology, ed 2. Churchill living- stone, Edinburgh, 1992 - Riggs, JE and Schocher, SS, Jr: Muscle Disease. Chapter 53. In Joynt, RJ (ed): Clinical Neurology, vol 4. JB Lippincott, Philadelphia, 1991]). Disorders in which muscle function is impaired by neural elements do not constitute myopathies. Under the heading "Metabolic Myopathies," we consider inborn errors of glycogen metabolism, lipid metabolism, and the mitochondrial respiratory chain (RC) ([DiMamo, S: Metabolic Myopathies. Chapter 10. In Adachi, M and Sher, JH(eds): Neuromuscular Dis- ease. 19aku-Shoin, New York, 1990]). Thus, metabolic myopathies are a group of genetic disorders that result from the inability of skeletal muscle to produce or maintain adequate levels of energy (ATP). These diseases are different from myopathies induced by medications that can impair muscle intermediary metabolism (i.e., statins[(Baker SK, Vladutiu GD, Peltier WL, et al. Metabolic myopathies discovered during investigations of statin myopathy. Can J Neurol Sci. 2008;35:94–97]), which are generally grouped within the category of toxic myopathies.

The first published case report of a ''metabolic myopathy,'' was described by McArdle in 1951 ([McArdle B. Myopathy due to a defect in muscle glycogen breakdown. Clin Sci. 1951;24:13–36]). Since then, the knowledge of the pathophysiology of metabolic myopathies has significantly improved and today an identification of the most common genetic and biochemical defects of these disorders is possible ([Hirano M, DiMauro S. Metabolic myopathies. Adv Neurol. 2002;88:217–234]).

The metabolic myopathies are generally classified according to the altered area of metabolism and can be divided into muscle glycogenoses, disorders of lipid metabolism and mitochondrial myopathies. To understand this diverse group of diseases, it is helpful to briefly review normal muscle physiology. During exercise, the working muscle needs a continuous supply of adenosine triphosphate (ATP), but the initial stores of ATP in the muscles are used up very quickly or preserved for other functions (i.e. electrochemical equilibrium). Therefore, ATP must be regenerated. The resynthesis of ATP involves three energy systems (Fig. 1).

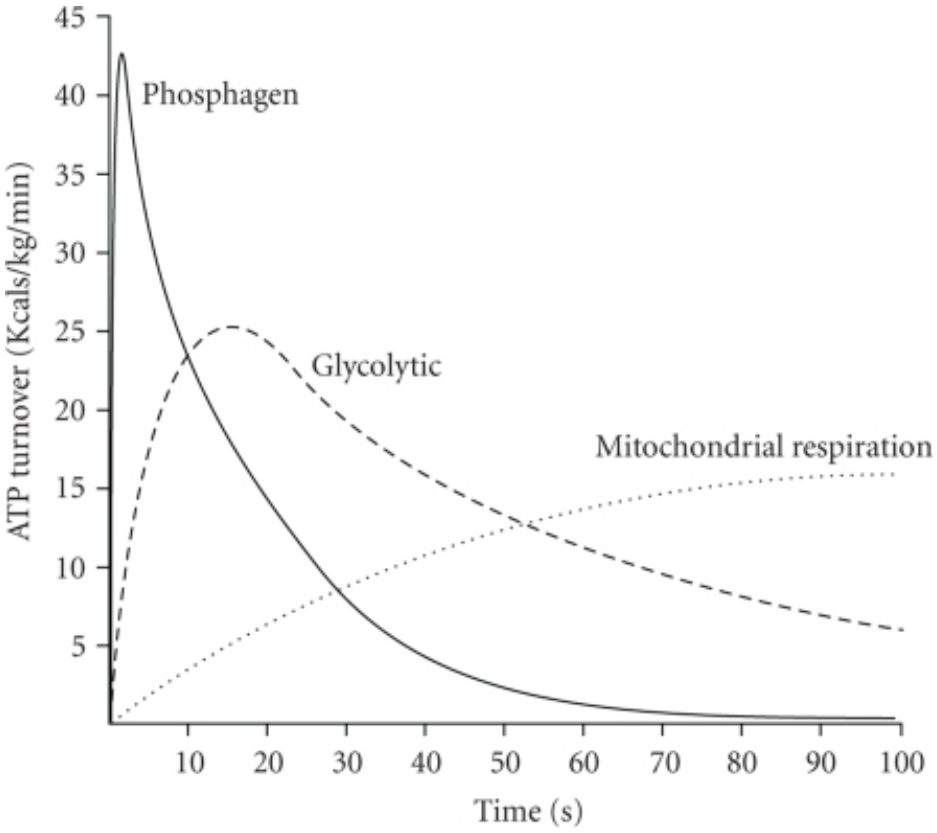

Figure 1. The three energy systems of muscle ATP regeneration.

The first energy source is the high-energy phosphagen system, which is characterized by the use of phosphocreatine (PCr) as a substrate. The initial concentrations of ATP and PCr, which are low and are depleted rapidly in high-intensity work in the muscle, are limiting factors for the high-energy phosphate system. Therefore, this system, which is able to produce very large amounts of energy in a short duration of time, can provide energy for muscles in the initial 1 to 15 s of high-intensity activity (Hultman E, Bergstrom J, Anderson NM (1967) Breakdown and resynthesis of phosphorylcreatine and adenosine triphosphate in connection with muscular work in man. Scand J Clin Lab Invest 19:56–66). This means that after this short period of time, ATP resynthesis must occur through the other two main energy systems: anaerobic glycolysis or aerobic oxidation. In anaerobic glycolysis, energy is released from the catabolism of muscle storage glycogen or blood glucose to pyruvate (Hargreaves M (2000) Skeletal muscle metabolism during exercise in humans. Clin Exp Pharmacol Physiol 27:225–228). Under anaerobic conditions, when the work output is over 25–30% of maximum voluntary contraction (MVC), glycogen represents the main source of ATP. The third energy source is the aerobic oxidative energy system, which is the main ATP supply during exercise activities performed for several minutes. Limiting factors for this system are sufficient tissue amount of mitochondria, oxygen availability, and the presence of the enzymes involved in the metabolic pathway. Blood lactate levels remain relatively low during purely aerobic exercise. During the recovery period after exercise, the oxidative metabolism is the only source for the re-

synthesis of PCr and ATP. Moreover, lactate is picked up from blood by type I fibers, where it is converted back to pyruvate, which enters the Krebs cycle (Fig. 2).

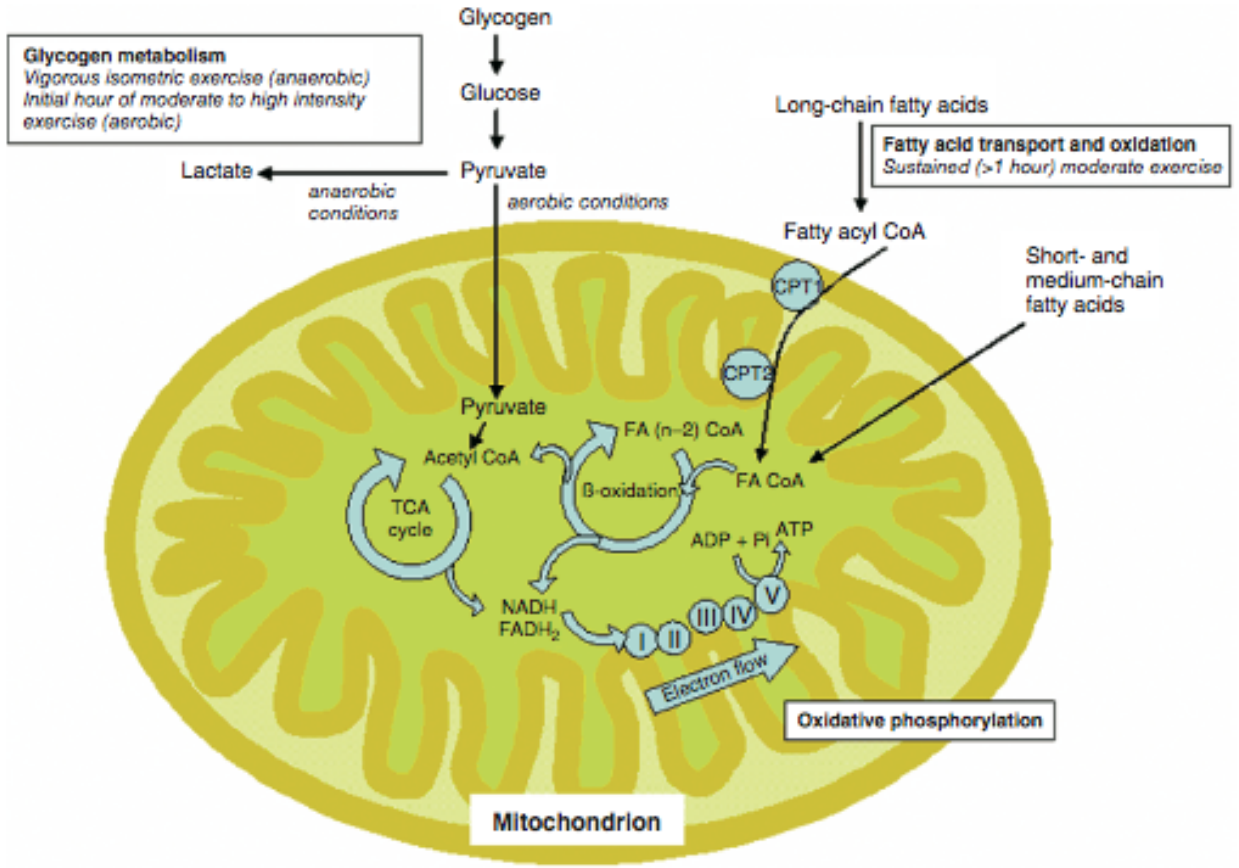

Figure 2. Skeletal muscle bioenergetics

Thus, metabolic myopathies include either disorders of glycogen/glucose metabolism or disorders of lipid metabolism whereas mitochondrial myopathies encompass disorders that impair both fat and carbohydrate metabolism and restrict oxidative phosphorylation. As for abnormalities of glycogen metabolism (Glycogenoses - GSD), several defects throughout the cascade of enzymes and substrates that split glycogen to pyruvate have been identified (Table 1). Moreover, there could also be defects in the glycogen degradation process. In most of these disorders skeletal muscle is affected to some extent (types V, VII, X, and XI). In other disorders the enzymatic defect is more generalized and symptoms arising in other systems predominate, especially in the liver.

Type	Defect	Associated clinical features	Laboratory features
Disorders causing reversible muscle symptoms such as exercise intolerance, myalgia, myoglobinuria			
GSD V McArdle	Myophosphorylase-M isoform	Limited to muscle 'Second wind' phenomenon Onset usually in childhood/adolescence	Minimal glycogen storage Many necrotic and regenerating fibres Mutations in *PYGM* gene (Arg50Stop most common in Caucasians)
GSD VII Tarui	Phosphofructokinase-M isoform	Presentation may be indistinguishable from McArdle disease Carbohydrate intake exacerbates exercise intolerance (out of wind phenomenon) Variant manifests as late onset progressive weakness	Mild haemolytic anaemia (raised bilirubin and reticulocytes) Muscle polyglucosan and glycogen deposition
GSD IXa/formerly VIII	Phosphorylase b kinase, α- and β-subunit	Muscle symptoms usually mild, often asymptomatic. Various types affecting liver (hepatomegaly), muscle, heart	Fasting hypoglycaemia, raised cholesterol and triglycerides if liver involved Phosphorylase kinase activity low
GSD X	Phosphoglycerate kinase, A-isoform Phosphoglycerate mutase, M-subunit	Haemolytic anemia, seizures, mental impairment Limited to muscle High frequency of manifesting heterozygotes	Accumulation of glycogen granules may be light Glycogen deposition, may be tubular aggregates. Muscle PGA mutase activity 5–7% of normal
GSD IX	LDH-A	Erythematous skin lesions in some cases	Raised creatine kinase with no corresponding LDH rise
GSD XIII	β-Enolase	Limited to muscle	Focal sarcoplasmic accumulation of glycogen- β particles. Dramatically reduced β-enolase protein
GSD 0	Glycogen synthase	Muscle fatigability, hypertrophic cardiomyopathy Causes similar problems to GSDs but no storage of glycogen	Lack of glycogen in muscle, predominance of oxidative fibres, mitochondrial proliferation
Disorders causing progressive myopathy and persistent weakness			
GSD II Pompe	Acid α-glucosidase (acid maltase)	Severe infantile form, fatal in first few years of life Late onset form resembles polymyositis or limb girdle muscular dystrophy Early respiratory involvement	Vacuolization on electron microscopy, lysosomal free glycogen deposition Reduced acid α-glucosidase in fibroblasts, muscle
GSD III Cori/Forbes	Amylo-1,6-glucosidase (debranching enzyme)	Hepatomegaly, hypoglycemia, failure to thrive, usually improves at puberty Some later develop distal myopathy	Accumulation of abnormal glycogen with short outer chains Vacuolization
GSD IV Andersen	Alpha-1,4-glucan branching (branching enzyme)	Hepatosplenomegaly, cirrhosis, liver failure. Cardiomyopathy. Isolated myopathy in some patients Affects Ashkenazi Jews	Reduced branching enzyme activity in red cells. Polyglucosan bodies in muscle
GSD XII	Aldolase A	Progressive weakness and episodic muscle symptoms. Haemolytic anaemia, mental retardation, short stature	Red cell aldolase activity less than 6% of normal
Formerly GSD IIb Danon	Mutations in LAMP2	Heart conduction defects, hypertrophic cardiomyopathy Mild muscle weakness, variable mental retardation	Lysosomal glycogen storage but with normal acid maltase activity. Vacuolization Staining for LAMP2 in cultured fibroblasts

GSD, glycogen storage disorder; LAMP, lysosome-assocaited membrane protein; LDH, lactate dehydrogenase; PFK, phosphofructokinase; PGA, phosphoglycerate.

Table 1. Disorders of glycogen metabolism affecting muscle (DiMauro S. Muscle glycogenoses: an overview. Acta Myol 2007; 26:35–41)

The diseases impairing lipid metabolism ultimately impair β-oxidation of lipid within the mitochondrial matrix. The main defects that have been identified include transport of long-chain fat across the mitochondrial membrane (i.e., CPT deficiency) or transport of carnitine into the cell (ie, carnitine transporter deficiency), and mutations in β-oxidation enzymes directly [ie, long-chain acyl-CoA dehydrogenase, medium-chain acyl-CoA dehydrogenase, trifunctional protein, deficiency]. The more severe variants present in infancy or childhood with a primary liver or encephalopathy picture, whereas the adult-onset forms are predominately myopathic (Tein I. Metabolic

myopathies. Semin Pediatr Neurol. 1996;3:59–98). Mitochondrial myopathies represent a diverse group of conditions with a primary defect in electron transport chain function (Fig. 3). These disease include myopathies caused by primary mtDNA mutations, nuclear mutations that lead to multiple mtDNA deletions or depletion, and nuclear mutations that impair the synthesis of individual respiratory chain subunits or mitochondrial protein synthesis (DiMauro, S., Schon, E. A., Carelli, V., et al. The clinical maze of mitochondrial neurology. Nat Rev Neurol. 2013; 9(8): 429– 444).

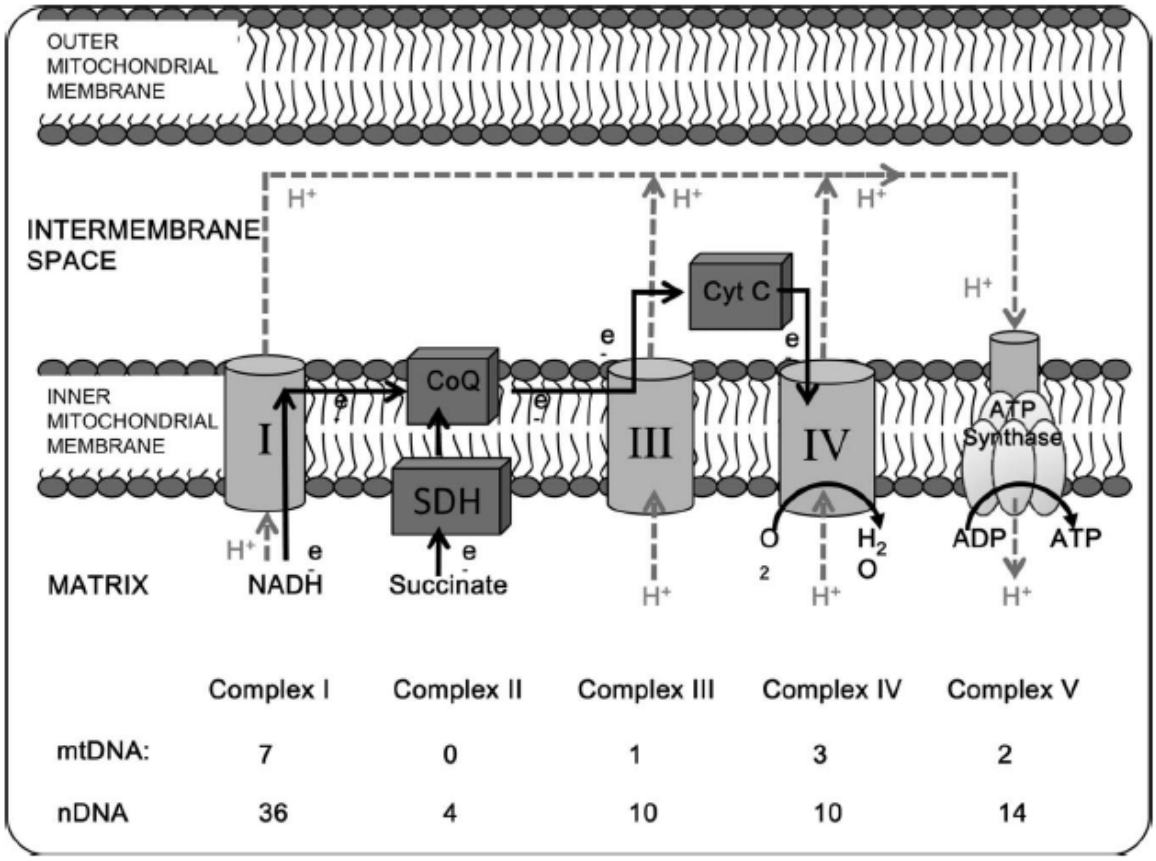

Figure 3. Schematic illustration of the mitochondrial electron transport chain and the relative contribution of subunits encoded by mtDNA and nuclear DNA (nDNA).

POMPE DISEASE

Pompe disease (GSD II or acid maltase deficiency) is an autosomal recessive disorder caused by deficiency of the lysosomal enzyme acid-α-glucosidase (GAA) which catalyzes the hydrolysis of α-1,4 and α-1,6 links of glycogen. The enzyme deficiency leads to lysosomal accumulation of glycogen and disruption of tissue architecture in different organs, especially the skeletal muscles, heart and liver. The continuum spectrum of clinical phenotypes ranges from an infantile-onset rapidly fatal form to a slowly progressive adult form (R. Hirschhorn, A.J. Reuser, Glycogen storage disease type II: acid alpha glucosidase deficiency, in: B.A. Scriver, W. Sly, D. Valle (Eds.), The Metabolic and Molecular Bases of Inherited Disease, 8th edition, McGraw-Hill, New York, 2001, pp. 3389–3420). The classic infantile-onset form, usually owing to complete GAA deficiency, presents in the first months of life with generalized marked hypotonia and muscle

weakness, severe cardiomyopathy, feeding difficulties, failure to thrive, and respiratory failure. Untreated patients die within the first year of life of cardiorespiratory dysfunction (P. Laforêt, M. Nicolino, P.B. Eymard, J.P. Puech, C. Caillaud, L. Poenaru, M. Fardeau, Juvenile and adult-onset acid maltase deficiency in France: genotype–phenotype correlation, Neurology 55 (8) (2000) 1122–1128). In the juvenile forms symptoms appear between 2 and 5 years of age, and cardiomyopathy is rarely seen. The adult forms, named late-onset GSDII and usually characterized by residual enzyme activity, are slowly progressive myopathies that leads to a progressive loss of motor function. Diaphragmatic weakness and respiratory insufficiency are frequent and induce early restrictive respiratory insufficiency (M.L. Hagemans, W.J. Hop, P.A. Van Doorn, A.J. Reuser, A.T. Van der Ploeg, Course of disability and respiratory function in untreated late onset Pompe disease, Neurology 66 (4) (2006) 581–583). Cardiac involvement is rarely reported in adult patients. When present, it is usually less severe than infantile patients and is characterized by cardiac hypertrophy (involving the left ventricular wall or the ventricular septum) and conduction abnormalities. In untreated late-onset patients, muscle strength and pulmonary function usually deteriorate over the years, leading to wheelchair use and respiratory support in most of cases; it has been demonstrated that untreated adult GSDII patients present an invariably progressive disease, have higher mortality compared with the general population, and present a poor quality of life (D. Gungor, J.M. de Vries,W.C. Hop, A.J. Reuser, P.A. van Doorn, A.T. van der Ploeg,M.L. Hagemans, Survival and associated factors in 268 adultswith Pompe disease prior to treatment with enzyme replacement therapy, Orphanet J. Rare Dis. 6 (2011) 34). Genetic testing and the blood spot enzymology testing serve as diagnostic tests, although a confirmatory muscle biopsy is sometimes required. The histopathological hallmark of Pompe disease is increased muscle fiber vacuolization and autophagy (Fig. 4).

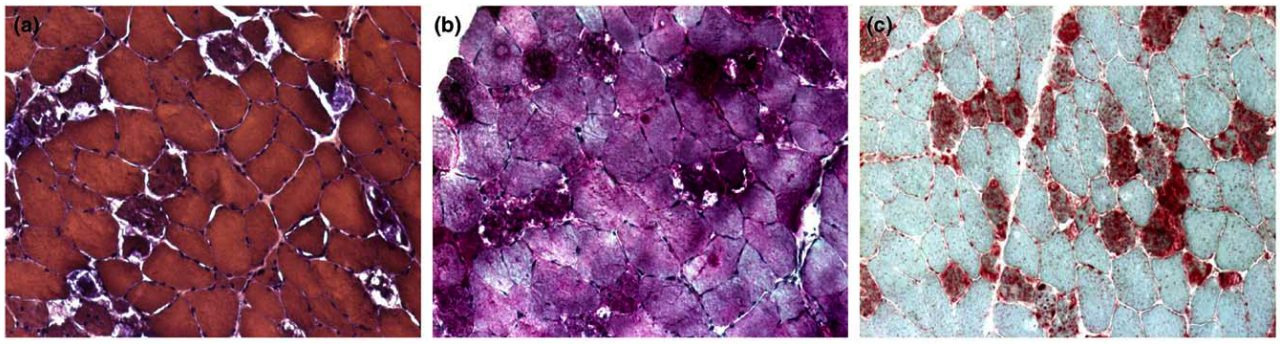

Figure 4. Muscle biopsy of a late-onset GSDII patient: (a) fiber vacuolization (H&E); (b) PAS-positive vacuoles (PAS reaction); (c) increased autophagy (acid phosphatase);

The vacuoles vary in size and shape and show PAS-positivity and strong reaction for lysosomal acid phosphatase. Most of the infantile and childhood-onset forms typically exhibit fibers occupied by huge vacuoles that contain basophilic amorphous PAS positive materials. In late-onset patients the degree of vacuolization is extremely variable and it appears sometimes independent of age of onset, disease duration, or clinical features. Acid phosphatase positive globular inclusions can be

found in a small proportion of patients with adult-onset Pompe disease, and have been proposed as a hallmark of Pompe disease and a useful diagnostic marker when typical vacuolated fibers are lacking ([Raben N, Roberts A, Plotz PH: Role of autophagy in the pathogenesis of Pompe disease. Acta Myol 2007, 26:45-48]). Genotype–phenotype correlations showed that the disease phenotype is hard to predict on the basis of gene mutations alone, in most cases, even if the nature of the mutation sometimes matches the clinical phenotype. A role of the genetic background and a modifying effect of exogenous factors on GAA gene expression is present and shifts the enzyme biosynthesis and degradation rate. Several enzyme replacement therapies (ERT) have been tried in GSDII patients since 1967, when the enzyme derived from Aspergillus niger was unsuccessfully tested ([Hug G, Schubert WK: Lysosomes in type II glycogenosis. Changes during administration of extract from Aspergillus niger. J Cell Biol 1967, 35:C1-C6]). The first effective approach to the ERT was the rhGAA (Myozyme) derived from hamster ovary cells. The treatment was soon demonstrated to be effective in infantile patients in markedly reducing left ventricular mass and improving cardiomyopathy, which are the hallmark of infantile form and the primary cause of death of infant patients. Furthermore, rhGAA treatment reduced the risk of death and of invasive ventilation, compared with an untreated control group ([P.S.Kishnani, M.Nicolino, T.Voit, R.C. Rogers,A.C. Tsai, J.Waterson,G.E.Herman, A. Amalfitano, B.L. Thurberg, S.Richards,M. Davison, D. Corzo, Y.T. Chen, Chinese hamster ovary cell-derived recombinant human acid a-glucosidase in infantile-onset Pompe disease, J. Pediatr. 149 (2006) 89–97]). In adult GSDII patients is difficult to define the impact of therapies due to the slowly progressing nature of the disease and the wide variability of organ involvement. The only randomized placebo-controlled study is the LOTS (Late Onset Treatment Study), in which 90 patients between 10 and 70 years of age were treated with rhGAA (60 patients) or placebo (30 cases) for 18 months ([A.T. van der Ploeg, P.R. Clemens, D. Corzo, D.M. Escolar, J. Florence, G.J. Groeneveld, S. Herson, P.S. Kishnani, P. Laforet, S.L. Lake, D.J. Lange, R.T. Leshner, J.E. Mayhew, C. Morgan, K. Nozaki, D.J. Park, A. Pestronk, B. Rosenbloom, A. Skrinar, C.I. van Capelle, N.A. van der Beek, M. Wasserstein, S.A. Zivkovic, A randomized study of alglucosidase alfa in late-onset Pompe disease, N. Engl. J. Med. 362 (15) (2010) 1396–1406]). The treatment improved walking distance measured with the 6-minute walk (6-MWT) test of 65 m and stabilized pulmonary function in the rhGAA group, as compared to the slight worsening of walked distance and respiratory function observed in the placebo group. Nevertheless other open-label studies evidenced a rather variable course of neuromuscular outcomes in adult onset GSDII patients during long observational periods of ERT, demonstrating a variable effect in prolonging walked distance and respiratory function. Thus, it is clear that ERT is effective in infantile form, especially for reducing cardiomegaly and prolonging children's survival. On the contrary, in late-onset GSDII it is not easy to demonstrate treatment efficacy both on the slowly progressive motor impairment and respiratory failure. The analysis of clinical efficacy in adult patients evidenced that the motor response is widely variable in each patient. For a better evaluation of natural course and treatment efficacy,

accurate quantitative outcome measures should be defined. Indeed, excluding the manual muscle testing for muscle strength, the examination of patients usually includes a series of functional tests, such as the 6-MWT, and the timed tests, such as the GSGC score (Gait, climbing Stairs, Gowers' maneuver and arise form a Chair), which are not very sensitive to underline small differences in the clinical course of treated and untreated patients (C. Angelini, C. Semplicini, S. Ravaglia,M.Moggio, G.P. Comi, O.Musumeci, E. Pegoraro, P. Tonin, M. Filosto, S. Servidei, L.Morandi,G. Crescimanno, G.Marrosu,G. Siciliano, T. Mongini, A. Toscano, Italian Group on GSDII, Newmotor outcome functionmeasures in evaluation of late-onset Pompe disease before and after enzyme replacement therapy, Muscle Nerve 45 (6) (2012) 831–834). An increasing role in the evaluation of muscle disease has been acquired by muscle MRI, a non-invasive exam that can evaluate muscle mass and fibro-fatty muscle degeneration, and, when associated with spectroscopic studies, it may measure glycogen content (R.Y. Carlier, P. Laforet, C.Wary, D.Mompoint, K. Laloui, N. Pellegrini, D. Annane, P.G. Carlier, D. Orlikowski,Whole bodymuscle MRI in 20 patients suffering from late onset Pompe disease: involvement patterns, Neuromuscul. Disord. 21 (2011) 791–799). The identification of the factors underlying the variability of clinical response to ERT needs further investigation. In the meantime, other therapeutical options have been tried. A dietary treatment with high-protein and low-carbohydrates, supplemented with L-alanine, and physical aerobic submaximal exercise was proposed (Slonim AE, Bulone L, Goldberg T, Minikes J, Slonim E, Galanko J, Martiniuk F: Modification of the natural history of adult-onset acid maltase deficiency by nutrition and exercise therapy. Muscle Nerve 2007, 35:70-77). The purpose was to decrease the deposition of glycogen in lysosomes, and to antagonize the muscle protein catabolism typical of GSDII patients, with the dietary treatment, and stimulate fatty acid utilization in muscles like energy resource with aerobic exercise. The clinical outcome in one study was surprisingly good, even if the compliance to the scheme was not easy, and the increase in the weight of some patients worsens their motor function.

McARDLE DISEASE

McArdle disease (Glycogenosis type V - GSD V), or myophosphorylase deficiency is a disorder of skeletal muscle carbohydrate metabolism first described by Brian McArdle in 1951 (McArdle B. Myopathy due to a defect in muscle glycogen breakdown. Clin Sci. 1951;10:20). It is one of the most frequent genetic myopathies and it is due to an autosomal recessive mutation in both copies of the gene PYGM, encoding the muscle isoform of glycogen phosphorylase, myophosphorylase (Bruno C, Cassandrini D, Martinuzzi A, et al. McArdle disease: the mutation spectrum of PYGM in a large Italian cohort. Hum Mut. 2006;27(7):718). Because myophosphorylase catalyzes and regulates the breakdown of glycogen into glucose-1-phosphate in muscle fibers, patients are unable to obtain energy from their muscle glycogen stores (Di Mauro S. Muscle glycogenoses: an overview. Acta Myol. 2007;26(1):35–41). Thus, glycolysis is blocked upstream and the muscle fibers of McArdle disease patients can take up glucose from the blood and convert it into glucose-6-phosphate, which then enters the downstream steps of glycolysis. Muscle glycolysis is not,

therefore, totally impaired in these patients. The reduction of pyruvate production in the tricarboxylic acid cycle, and the subsequent oxidative phosphorylation impairment bring to reduction of AcetylCoA production also with a worsening of function of the breakdown of fatty acid. Exercise intolerance is present virtually in all patients and often starts during childhood. For example, 58 % of patients reported that symptoms started in the first decade of life, but others reported that they appeared later in life (i.e. 28 % in the second decade and 14 % in the third or fourth decade) ([Lucia A, Ruiz JR, Santalla A, et al. Genotypic and phenotypic features of McArdle disease: insights from the Spanish national registry. J Neurol Neurosurg Psychiatry. 2012;83(3):322–8]). Exercise intolerance typically consists of acute crises of early fatigue and muscle stiffness and contractures, especially at the start of exercise, which usually disappear if exercise is stopped or intensity is reduced (Fig. 5).

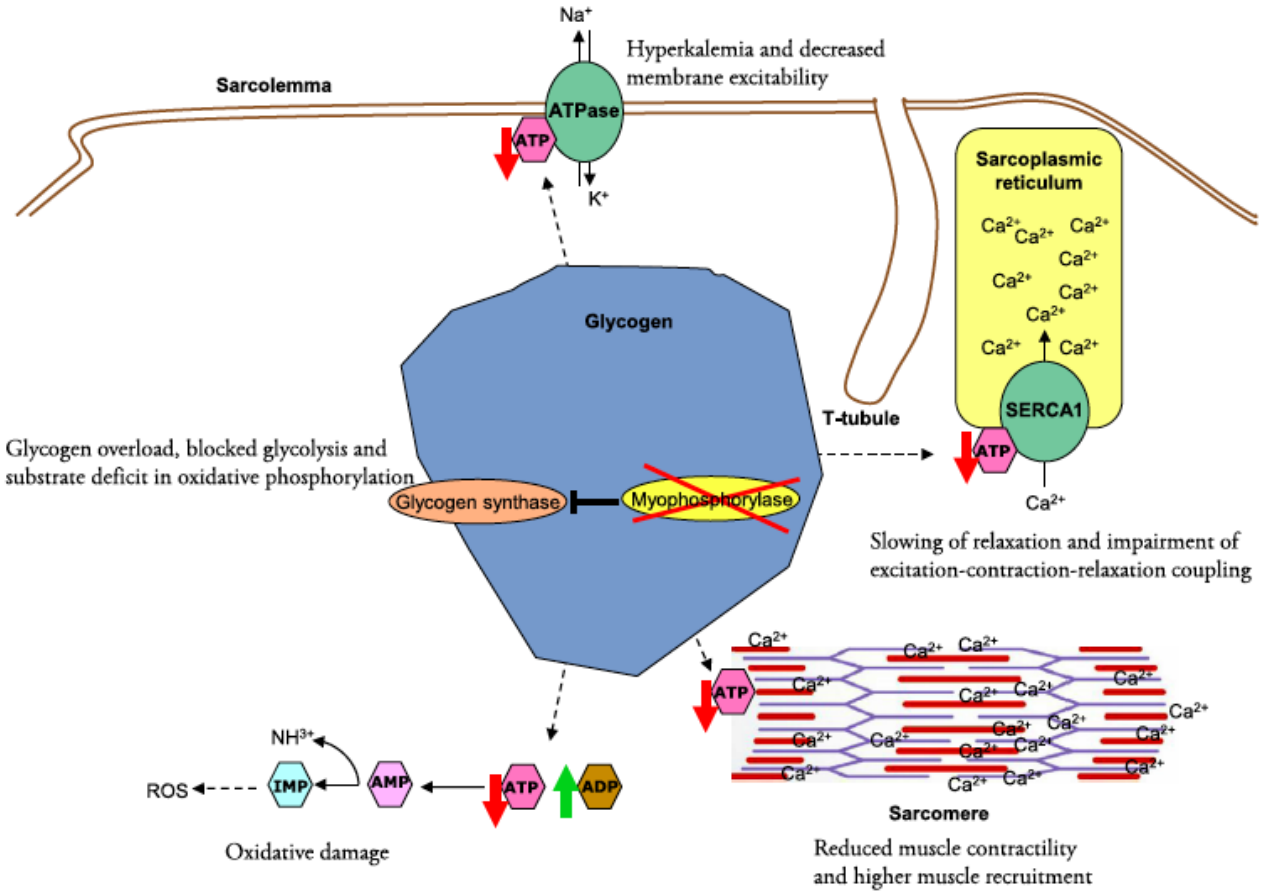

Figure 5. The pathophysiology of exercise intolerance and muscle damage in McArdle disease.

A unique feature of the disease is the so-called 'second wind' phenomenon, which most patients refer to as the ability to resume dynamic, large-mass exercise (e.g. brisk walking) if they take a brief rest upon the appearance of premature fatigue early in exercise ([Haller RG, Vissing J. Spontaneous, ''second wind'' and glucoseinduced second ''second wind'' in McArdle disease: oxidative mechanisms. Arch Neurol. 2002;59(9):1395–402]). The first few minutes of exercise act as a warm-up (e.g. inducing muscle vasodilation), after which more circulating free-fatty acids as well as glucose are available to working muscle fibers that can oxidize

these substrates, leading to attenuation of exercise intolerance. However, in these patients episodes of marked muscle damage or rhabdomyolysis are not rare. Thus, high serum CK activity (typically[1,000 U/L) triggered by exercise is a common finding which can be accompanied by myoglobinuria, typically referred to as 'dark urine' (Di Mauro SHA, Tsujino S. Nonlysosomal glycogenoses. In: Engel AGFAC, editor. Myology. New York: McGraw-Hill; 2004. p. 1535–58). The main potential danger of exertional rhabdomyolysis is acute renal failure as well as hyperkalemia, with the former eventually leading to chronic renal failure, although reported cases of life-threatening situations are very scarce. The intensity of the stimuli causing intolerance also shows inter-individual variability; in some rare cases (fittest patients) symptoms are triggered only by sports participation, whereas most people show intolerance to almost all types of physical exercise, and 25 % of patients also report functional limitations during daily life activities such as household tasks, personal care, lifting/carrying weights during shopping, or carrying children (Lucia A, Ruiz JR, Santalla A, et al. Genotypic and phenotypic features of McArdle disease: insights from the Spanish national registry. J Neurol Neurosurg Psychiatry. 2012;83(3):322–8). Patients often show an abnormally high cardiac output and heart rate at a given workload during dynamic exercise (Grassi B, Porcelli S, Marzorati M, Lanfranconi F, Vago P, Marconi C, Morandi L. Metabolic myopathies: functional evaluation by analysis of oxygen uptake kinetics. Med Sci Sports Exerc 41: 2120–2127, 2009). This 'hyperkinetic' circulation could be mediated by the local effects of potassium, inorganic phosphate, or adenosine, or a combination of these substances, released excessively from working skeletal muscles on metabolically sensitive skeletal muscle afferents and vascular smooth muscle (Haller RG, Lewis SF, Cook JD, et al. Hyperkinetic circulation during exercise in neuromuscular disease. Neurology. 1983;33(10):1283–7). The abnormal cardiovascular response to dynamic exercise in these patients could also be due, at least in part, to an altered central motor pattern, which may manifest as exaggerated motor unit recruitment for a given workload. As for impaired sympathetic nervous system activity, previous research has shown that these patients have normal muscle sympathetic nerve responses to exertion, at least for static (handgrip) exercise (Vissing J, Vissing SF, MacLean DA, et al. Sympathetic activation in exercise is not dependent on muscle acidosis. Direct evidence from studies in metabolic myopathies. J Clin Invest. 1998;101:1654–60). Despite symptoms of exercise intolerance from early childhood, the diagnosis is almost never made in the first decade of life; in approximately 50% of cases, the diagnosis is delayed until the fourth decade or later (Lucia A, Ruiz JR, Santalla A, et al. Genotypic and phenotypic features of McArdle disease: insights from the Spanish national registry. J Neurol Neurosurg Psychiatry 2012;83(3):322–8). Generally, diagnostic testing is undertaken only after a sentinel event, such as an episode of rhabdomyolysis and myoglobinuria or after the discovery of an otherwise unexplained elevated serum CK level, which commonly is 5 to 10 times the upper limit of normal. Recognition of the characteristic history of exercise intolerance is key to undertaking appropriate diagnostic testing. Non-ischemic forearm exercise testing is recommended (instead of ischemic exercise, which triggers a muscle contracture)

to demonstrate the block in glycogenolysis by absent lactate and increased ammonia production. The final diagnosis is usually reached by routine phosphorylase histochemistry and genetic testing on muscle biopsy.

Many drugs or dietary treatments have been tested to alleviate symptoms in this rare disease, but most of them failed to demonstrate a significant amelioration or were not well tolerated. No significant beneficial effects have been reported in McArdle disease patients receiving branched chain amino acids supplementation or fatty acid-rich diet to provide alternative energy sources to glycolytic metabolism. High-dose oral ribose (Steele IC, Patterson VH, Nicholls DP. A double blind, placebo controlled, crossover trial of D-ribose in McArdle's disease. J Neurol Sci. 1996;136(1–2):174–7) or vitamin B6 (Phoenix J, Hopkins P, Bartram C, et al. Effect of vitamin B6 supplementation in McArdle's disease: a strategic case study. Neuromuscul Disord. 1998;8(3–4):210–2) failed to improve exercise tolerance in McArdle patients. More controversial are the results for creatine supplementation: low-dose supplementation (60 mg/kg/day for 4 weeks) attenuated muscle complaints in five of nine McArdle disease patients tested, but higher doses (150 mg/kg/day) increase intracellular phosphocreatine and actually increased exercise-induced myalgia (Vorgerd M, Zange J, Kley R, Grehl T, Hu¨ sing A, Ja¨ ger M, Mu¨ ller K, Schro¨ der R, Mortier W, Fabian K et al.: Effect of high-dose creatine therapy on symptoms of exercise intolerance in McArdle disease: double-blind, placebo-controlled crossover study. Arch Neurol 2002, 5:97-101). A beneficial intervention for alleviating exercise intolerance symptoms and 'protecting' the muscle from rhabdomyolysis risk consists of ensuring that sufficient blood glucose (derived from high hepatic glycogen stores) is constantly made available to patients' muscles during the daytime. This can be effectively achieved by adopting a diet with a high proportion (65%) of complex carbohydrates (such as those found in vegetables, fruits, cereals, bread, pasta and rice) and a low proportion (20 %) of fat [132]. Another strategy is the ingestion of simple carbohydrates before engaging in strenuous exercise: (a) in adults, 75 g of sucrose 30–40 min pre-exercise (Vissing J, Haller RG. The effect of oral sucrose on exercise tolerance in patients with McArdle's disease. N Engl J Med. 2003;349(26):2503–9), or lower doses (30–40 g of glucose, fructose or sucrose, which translates to 400–500 mL of most commercially available sport drinks) closer to the start of exertion (Andersen ST, Haller RG, Vissing J. Effect of oral sucrose shortly before exercise on work capacity in McArdle disease. Arch Neurol. 2008;65(6):786–9); and (b) in children, 20 g during the warm-up period preceding any vigorous exercise bout (Perez M, Mate-Munoz JL, Foster C, et al. Exercise capacity in a child with McArdle disease. J Child Neurol. 2007;22(7):880–2). Since individual differences in patients' physical activity levels seem to explain the heterogeneity in disease severity, it has been hypothesized that exercise training can reduce exercise intolerance of McArdle patients. Although evidence from randomized controlled studies is still missing, there is data from interventional research showing that patients with McArdle disease, adapt favorably to regular exercise and show an amelioration of clinical symptoms (Haller RG, Wyrick P, Taivassalo T, Vissing J (2006) Aerobic conditioning: an effective

therapy in McArdle's disease. Ann Neurol 59:922–928; Mate-Munoz JL, Moran M, Perez M et al (2007) Favorable responses to acute and chronic exercise in McArdle patients. Clin J Sport Med 17:297–303). It must be considered that vigorous dynamic ('aerobic') exercise should only be performed by the more habituated patients, while intense exercises involving high loads on low muscle mass should be generally discouraged. Moreover, pre-exercise carbohydrate ingestion might be suggested in order to attenuate the feelings of early fatigue and discomfort during the first minutes of a training session.

MITOCHONDRIAL MYOPATHIES

Mitochondrial myopathies have an estimated prevalence of 10 to 15 cases per 100,000 persons and are genetically determined disorders that result from a primary defect of the mitochondrial respiratory chain (Fig. 6). The number of defects responsible is experiencing exponential growth and now includes myopathies caused by primary mtDNA mutations or nuclear mutations that disrupt the function of individual respiratory chain subunits (DiMauro S, Schon EA, Carelli V, et al. The clinical maze of mitochondrial neurology. Nat Rev Neurol 2013;9(8):429–44). mtDNA mutations involves genes that encode polypeptide subunits of the respiratory chain or transfer or ribosomal RNAs that mediate the synthesis of entire mitochondrial proteins (DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. N Engl J Med 2003;348:2656–2668). These mtDNA mutations are usually inherited through maternal transmission and are thus expressed in a heteroplasmic fashion in cells and tissues, impairing respiratory chain function only when the percentage of mutant mtDNA reaches a critical threshold. Mutations in nuclear genes usually affect the central or peripheral nervous systems in addition to muscle function impairment and cardiac dysfunction is also often present. In the minority of cases it is possible to identify a well-defined clinical syndrome such as Kearns-Sayre syndrome (KSS), mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes (MELAS), myoclonic epilepsy with ragged red fibers (MERRF) or mitochondrial neuro-gastrointestinal encephalopathy (MNGIE) disease.

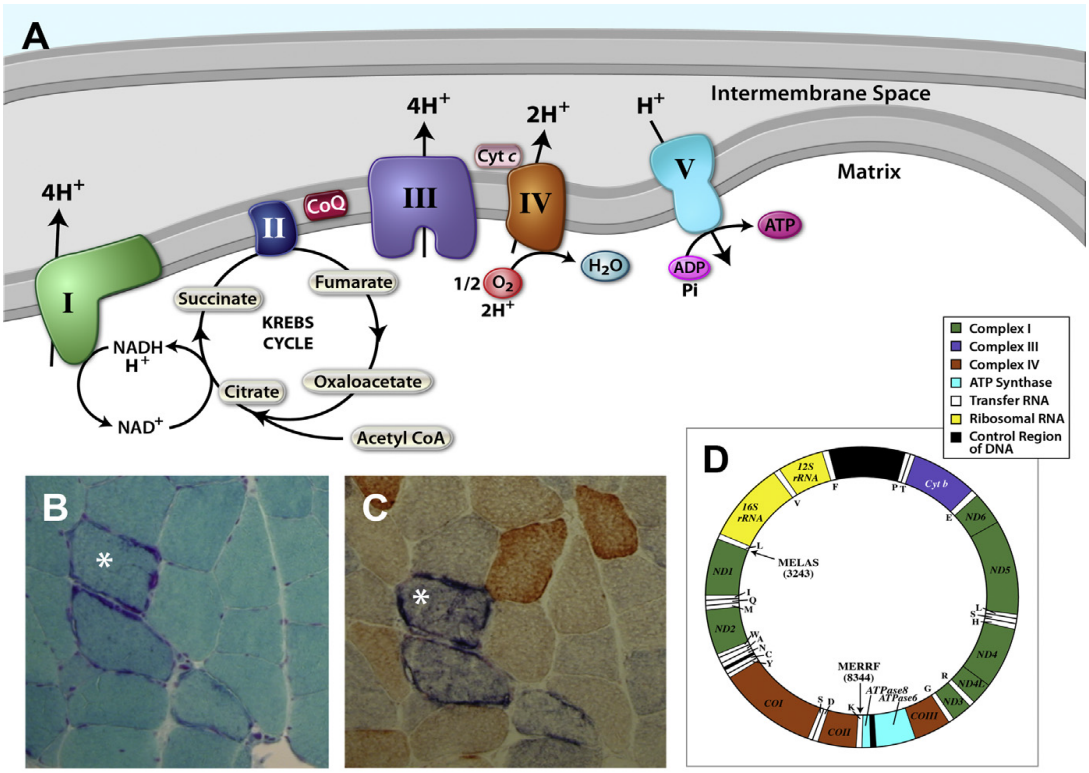

Figure 6. Mitochondrial respiratory chain complexes (A) and mitochondrial DNA (D). (B) and (C) are serial sections of muscle from a patient with a heteroplasmic mtDNA mutation.

Clinical features of these disorders include a proximal myopathy, stroke-like episodes, seizures, ataxia, cognitive decline, axonal neuropathy, sensorineural hearing loss, hypertrophic cardiomyopathy, pigmentary retinopathy, diabetes mellitus, short stature, and renal tubular acidosis. A number of diverse syndromes are characterized by specific combinations of these clinical features. Predominant involvement of one system can also occur. Muscle involvement is present in the majority of mitochondrial diseases and varied in its clinical presentation. Chronic progressive external ophthalmoplegia and eyelid ptosis often precede or accompany the skeletal muscle disease (Nardin RA, Johns DR. Mitochondrial dysfunction and neuromuscular disease. Muscle Nerve 2001;24:170–191). Mild weakness of the proximal limb musculature is usually present and is made worse by exertion. Patients often note myalgias and premature fatigue during exercise. Headache and nausea may occur during strenuous activity. More severe defects of oxidative phosphorylation result in a disparity between oxygen delivery and oxygen utilization and a hyperdynamic cardiopulmonary response to exercise. Patients thus experience marked tachycardia and exertional dyspnea when they engage in submaximal exercise. The age at onset of symptoms ranges from birth to late life, but is usually childhood or early adult life. Serum CK levels are normal or only mildly elevated. Electromyography usually shows mild myopathic or neuropathic changes, or a combination of both. An elevated resting and fasting lactate level (> 2.5 mmol/L) in the blood has high specificity but only modest sensitivity for the diagnosis (Tarnopolsky MA, Raha S. Mitochondrial myopathies: diagnosis, exercise intolerance, and treatment options. Med Sci Sports

Exerc 2005;37:2086–2093). Exercise testing using a cycle ergometer typically shows a reduction in maximal whole body oxygen consumption due to a reduction in peripheral oxygen extraction and a disproportionately greater production of carbon dioxide relative to oxygen consumption (Grassi B, Porcelli S, Marzorati M, Lanfranconi F, Vago P, Marconi C, Morandi L. Metabolic myopathies: functional evaluation by analysis of oxygen uptake kinetics. Med Sci Sports Exerc 41: 2120–2127, 2009). Muscle biopsy is required for specific diagnosis and it is typically performed from a limb muscle, such as the quadriceps femoris or deltoid. The major histochemical diagnostic feature is the presence of fibers deficient in cytochrome c oxidase activity (COX; complex IV of the respiratory chain), which represents low COX activity. COX-negative fibers are best identified by serially staining muscle for COX followed by SDH, which stains for complex II. The demonstration of COX-deficient, SDH-positive muscle fibers may have the best sensitivity and specificity for mitochondrial disease, particularly in adults. The subsarcolemmal accumulation of mitochondria, demonstrated by SDH histochemistry (ragged blue fibers), is another classical feature of mitochondrial myopathy.

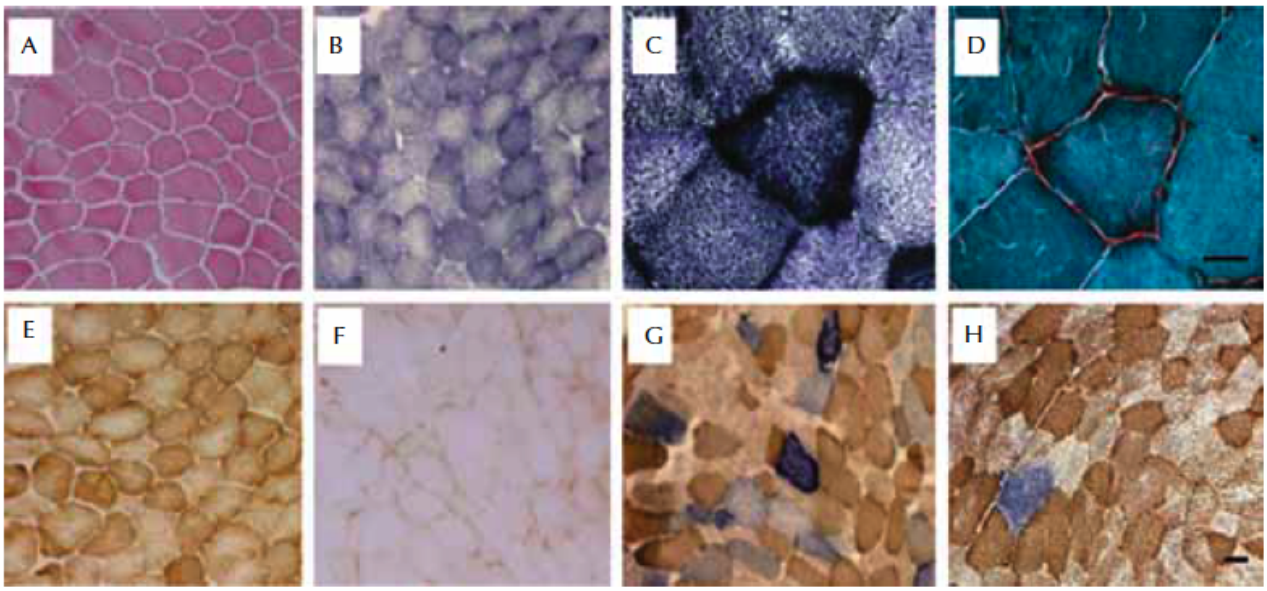

Figure 7. Muscle pathology in mitochondrial disorders. 20-μm cryostat sections of quadriceps skeletal muscle. (A) Hematoxylin and eosin, showing increased angulation of the muscle fibers and an increase in the proportion of internal nuclei. (B) Succinate dehydrogenase (SDH) histochemistry in a patient with a heteroplasmic mtDNA defect, showing subsarcolemmal proliferation of mitochondria. (C) Higher power view of SDH histochemistry showing a classical ragged red fiber. (D) Ragged red fiber shown by Gomori trichrome staining. (E) Cytochrome c oxidase (COX) histochemistry in a normal subject. (F) Global reduction in COX activity seen in a patient with a nuclear gene defect. (G) Mosaic COX defect demonstrated by sequential COX-SDH histochemistry in a patient with a heteroplasmic pathogenic mtDNA mutation. (H) COX-SDH histochemistry from an aged subject showing a single COX-deficient muscle fiber (based on Greenfields Pathology, 9th Edition).

Electron microscopy (EM) of muscle may demonstrate enlarged pleiomorphic mitochondria and paracrystalline inclusions. However, it provides minor support for the diagnosis of mitochondrial

disease because the findings are often nonspecific. Diagnostic information can also be obtained from respiratory chain enzyme analysis. Demonstrating a defect is an important diagnostic step in patients with normal or near-normal muscle histochemistry, particularly children. Biochemical assessment of respiratory chain function in muscle tissue is often coupled with molecular genetic studies ([Taylor RW, Schaefer AM, Barron MJ, McFarland R, Turnbull DM. The diagnosis of mitochondrial muscle disease. Neuromuscul Disord 2004;14:237–245]). The standard approach is to screen for common mtDNA point mutations using allele-specific assays or targeted sequencing, and to screen for mtDNA deletions using long-range polymerase chain reaction, or Southern blotting. Mutations in nDNA, when present, are always detectable from leucocyte DNA.

As for treatments, a recent Cochrane systematic review of all case series and treatment trials in mitochondrial diseases concluded that there were no treatments of proven benefit to influence the outcome of the disorder ([Pfeffer G, Majamaa K, Turnbull DM, Thorburn D, Chinnery PF. Treatment for mitochondrial disorders. Cochrane Database Syst Rev. 2012 Apr 18;4:CD004426]). Thus, the clinical management of mitochondrial disorders concentrates on supportive therapy, and symptomatic management of disease complications, such as those mentioned in the preceding section. Therapeutic agents to date have focused on various nutritional supplements, including carnitine, creatine, CoQ10, cysteine, dichloroacetate, dimethyglycine, and the combination of creatine, CoQ10, and lipoic acid, which have been evaluated in controlled trials. Various other agents including ascorbate and menadione, high-fat diet, magnesium, nicotinamide, and succinate have been of benefit in case reports, but further study would be required to indicate whether they are beneficial. Although there are no specific trials showing objective evidence of clinical efficacy, patients with a predominantly myopathic presentation should have muscle CoQ10 measurements performed, because patients with CoQ10 biosynthetic defects may respond to CoQ10 supplementation ([Rodriguez MC, MacDonald JR, Mahoney DJ, Parise G, Beal MF, Tarnopolsky MA. Beneficial effects of creatine, CoQ10, and lipoic acid in mitochondrial disorders. Muscle Nerve. 2007 Feb;35(2):235–242]). For patients with myopathy, there is evidence that various forms of exercise therapy are beneficial for numerous endpoints, including strength, fatigue, and quality of life. Aerobic endurance ([Taivassalo T, Gardner JL, Taylor RW, Schaefer AM, Newman J, Barron MJ, et al. Endurance training and detraining in mitochondrial myopathies due to single large-scale mtDNA deletions. Brain. 2006 Dec;129(Pt12):3391–3401]), and resistance ([Murphy JL, Blakely EL, Schaefer AM, He L, Wyrick P, Haller RG, et al. Resistance training in patients with single, large-scale deletions of mitochondrial DNA. Brain. 2008 Nov;131(Pt 11):2832–2840]) training programs have been studied. Exercise therapy may simply reverse the deconditioning, which is a common feature of many muscle diseases, or it is possible the exercise affects the underlying pathology, or a combination of these.

CINICAL EVALUTATION OF METABOLIC MYOPATHIES

The most important component of evaluating patients with a suspected myopathy is obtaining a comprehensive medical history. Because many myopathies are inherited, obtaining a thorough family history is of tremendous relevance in making a correct diagnosis. A detailed family tree should be completed to evaluate for evidence of autosomal dominant, autosomal recessive, and X-linked patterns of transmission.

Symptoms of muscle disease can be divided into negative complaints, such as exercise intolerance, fatigue, muscle atrophy and weakness, and positive complaints, such as contractures, cramps, myalgias, muscle stiffness, and myoglobinuria. Weakness is by far the most common negative symptom reported by patients with muscle disease. When the upper extremities are involved, patients notice trouble brushing their teeth, combing their hair, or lifting objects overhead. If the weakness involves the lower extremities, patients will complain of difficulty arising from a low chair or toilet, getting up from a squatted position, or climbing stairs. These symptoms in the arms and legs indicate proximal muscle weakness, which is probably the most common distribution of weakness in a myopathic disorder. Fatigue is a much less useful negative symptom because it may be a result of patients' overall health, cardiopulmonary status and level of conditioning. However, it is important to define the intensity and duration of exercise that provokes the fatigue. Myalgias may be episodic and may be associated to muscle cramps. Myoglobinuria is caused by the excessive release of myoglobin from muscle during periods of rapid muscle destruction (rhabdomyolysis) and is a relatively uncommon manifestation of muscle disease. Severe myoglobinuria can result in renal failure caused by acute tubular necrosis. It is obviously important to determine the onset, duration, and evolution of these symptoms and signs. Moreover, a history of precipitating factors that might trigger or exacerbate symptoms of weakness or myotonia should be explored. Involvement of organs or tissues other than muscle may also provide helpful clues in making the appropriate diagnosis. For example, respiratory failure may be the presenting symptom of acid maltase deficiency.

The laboratory investigation should include blood and urine tests, the forearm ischemic exercise test, electromyography, muscle biopsy, and molecular studies.

Blood and Urine Testing. Abnormal levels of specific compounds in the blood or urine, either alone or in combination, may help diagnose or suggest a specific type of metabolic abnormality. Tests primarily include CK, lactate, pyruvate, blood glucose and myoglobin. The CK level should be tested at rest and also during episodes of acute reversible muscle dysfunction with or without myoglobinuria. In patients with glycogen defects the CK level may be elevated at rest, particularly in patients with static features. By comparison, in other patients the CK can be normal between

acute episodes. Blood lactate and pyruvate may be elevated in patients with mitochondrial myopathies; the lactate/pyruvate ratio is useful in determining the site of enzymatic block in the pathways of mitochondrial metabolism. The determination of serum and urine myoglobin is important in patients with suspected rhabdomyolysis. Patients with acute myoglobinuria may have concurrent elevation of serum creatinine, potassium, phosphate, uric acid (myogenic hyperuricemia), or even serum amino acids (particularly taurine). In the patient with a suspected lipid metabolism defect, levels of plasma total and free carnitine, serum free fatty acids and ketones, serum acylcarnitines, urine acylglycines, and organic acids should be determined. The testing should preferably be performed during episodes of acute catabolic crises before glucose administration or during periods of fasting because normal values may be observed when the patient is metabolically stable and not fasting.

Electromyography. In patients with a fixed weakness, electromyography can be useful in excluding a neuropathic process and providing evidence for a myopathic condition. Myotonic discharges are evident in patients with myophosphorylase or acid maltase enzyme deficiency. In patients with excessive fatigability, repetitive nerve stimulation could be instrumental in excluding a defect in neuromuscular transmission (Griggs R, Mendell J, Miller R. Metabolic myopathies. In: Griggs R, Mendell J, Miller R, eds. Evaluation and treatment of myopathies. Philadelphia: F.A. Davis, 1995:247-93).

Six minute walking test. The six minute walking test (6MWT) is a useful measure of functional capacity, targeted at people with reduce exercise tolerance. It has been widely used for measuring the response to therapeutic interventions for patient management and research in patients with moderate-to-severe cardiopulmonary. The 6MWT is a test of relatively low complexity that measures the distance that a patient can quickly walk in a period of 6 min, referred to as the 6-min walk distance (6MWD). A change in 6MWD is usually reported as an absolute value in meters or feet. Alternatives are percentage change or change in percentage predicted. Besides it has emerged as the most commonly used test for the objective assessment of functional exercise capacity, statistically significant differences in groups from clinical trials are usually much smaller than a clinically important change in an individual patient.

Forearm Ischemic Exercise Test. If the clinical evaluation and laboratory findings suggest an enzymatic defect in the nonlysosomal glycogenolytic pathway and also in glycolysis, then the forearm ischemic exercise test should be performed. This test can be useful in assessing all patients with exercise intolerance (DiMauro S, Tsujino S. Nonlysosomal glycogenoses. In: Engel A, Banker B, eds. Myology. New York: McGraw-Hill, 1994:1554-76), although its use in younger children is limited. A needle should be placed in a superficial antecubital vein, and resting blood samples should be obtained for determination of serum lactate, pyruvate, CK, and ammonia levels. The blood pressure cuff should be inflated to a

pressure level above the systolic pressure, and the patient should be asked to perform one per second hand grips at least 75% of his maximum voluntary hand grip. If an acute cramp develops, the cuff should be immediately deflated. Some recommend that the test be performed without the blood pressure cuff in place; others inflate the cuff at a value intermediate between the systolic and diastolic blood pressures. If the patient tolerates the test and exercises adequately, blood samples should be obtained at intervals of 1, 2, 3, 5, and 10 minutes (after removal of the blood pressure cuff), for determination of the serum lactate, pyruvate, and ammonia levels; a single sample should be obtained for CK. Basal blood lactate values can be increased in patients with mitochondrial disorders or glycogenosis type 1 (Livingstone C, Chinnery PF, Turnbull DM (2001) The ischaemic lactate-ammonia test. Ann Clin Biochem 38:304–310). In normal individuals after a good effort a threefold to fivefold rise in lactate is evident within the first 1-3 minutes. The rise in serum ammonia is similar but somewhat slower and more gradual, reaching a peak at 3-4 minutes. When the glycogenolytic or glycolytic pathway is blocked, as McArdle disease, the ischemic forearm exercise test reveals failed lactate production with normal or exaggerated ammonia concentration increase (Sinkeler SP, Wevers RA, Joosten EM, Binkhorst RA, Oei LT, Van't Hof MA, De Haan AF (1986) Improvement of screening in exertional myalgia with standardized ischemic forearm test. Muscle Nerve 9:731–737). Patients with myophosphorylase deficiency may exhibit an abnormally high rise in ammonia.

Aerobic exercise test. Exercise testing can be a useful ancillary tool, especially to better characterize muscle fatigue and exercise intolerance referred by the patients. The main exercise tests used for diagnostic purposes in patients are performed with lactate determination during cycle or treadmill ergometry (Schmidt M, Kunkel M, Schuff-Werner P, Naumann M, Reichmann H, Reimers CD (1997) Standardised aerobic treadmill ergometry in healthy subjects and patients with mitochondrial and non-mitochondrial myopathies. Nervenarzt 68:831–835). No unique standardized protocols, at the moment, have been established. The aerobic exercise test can be performed following an incremental cycle test (Siciliano G, Rossi B, Manca L, Angelini C, Tessa A, Vergani L, Martinuzzi A, Muratorio A (1996) Residual muscle cytochrome c oxidase activity accounts for submaximal exercise lactate threshold in chronic progressive external ophthalmoplegia. Muscle Nerve 19:342–349) or a cycle test with a fixed constant absolute workload (Vissing J, Haller RG. A diagnostic cycle test for McArdle's disease. Ann Neurol 54: 539–542, 2003). In the skeletal muscle of patients with metabolic myopathies, a limited ability to increase the extraction of O_2 from blood relative to the increase in O_2 delivery by the circulation during exercise has been described (Grassi B, Marzorati M, Lanfranconi F, Ferri A, Longaretti M, Stucchi A, Vago P, Marconi C, Morandi L. Impaired oxygen extraction in metabolic myopathies: detection and quantification by near-infrared spectroscopy. Muscle Nerve 35: 510–520, 2007).

Muscle Biopsy. Given the impracticality of testing muscle biopsy tissue for all known metabolic defects, the initial clinical and laboratory assessment helps target the immunohistochemical and biochemical testing of the muscle tissue. The microscopic examination of the muscle sample

should include electron microscopy and immunohistochemical staining using various antibodies (e.g., phosphorylase, phosphofructokinase, cytochrome-c oxidase, and myoadenylate deaminase) if deficiencies of the respective enzymes are diagnostic possibilities. Microscopic examination will determine the presence or absence of glycogen or lipid storage or ragged–red fibers, such as in mitochondrial myopathies. Because these evaluations will be normal in a number of metabolic defects, additional biochemical evaluation of the muscle tissue may be pursued; the evaluation should focus on specific possible biochemical defects (e.g., assays of glycogenolytic/glycolytic enzymes, electron transfer chain complexes, and FAO enzymes) on the basis of the results of the preliminary noninvasive evaluations.

Molecular Studies. Specific defects can be characterized at the molecular level either by Western blotting or by molecular analysis of specific mutations. Western blotting can be used to differentiate between a kinetic deficiency and a defect in the production of the relevant enzyme. The identification of specific mutations (e.g., phosphorylase gene and mitochondrial genome mutations) can be used to precisely and rapidly detect specific defects and also to perform presymptomatic, prenatal, and carrier detection.

FUNCTIONAL EVALUATION OF OXIDATIVE METABOLISM

Most activities of daily living, such as rising from a chair, opening a jar, lifting a box, or walking at a slow pace, require only a modest amount of muscle strength or endurance, and do not involve significant demands on the respiratory or cardiovascular systems. However, vigorous aerobic exercises, such as running or sustained stair climbing, require tight integration of multiple systems in the body including the respiratory, cardiovascular, and neuromuscular systems. During physical exercise, adequate interactions among these systems are required to transport an adequate amount of oxygen and nutrients to the exercising muscles as well as to remove the metabolically produced carbon dioxide from the exercising muscles, to maintain homeostasis. Accordingly, each of these systems has important functions. The respiratory system, for example, is a ventilatory pump, moving oxygen from the atmosphere to the alveoli and carbon dioxide from the alveoli to the atmosphere. It must also provide an effective means of exchanging oxygen and carbon dioxide across the thin alveolar walls. The heart is responsible for pumping oxygenated blood to the exercising muscles as well as returning oxygen-poor and carbon dioxide-rich blood to the gas-exchanging surfaces of the lungs. Finally, the muscles must extract oxygen from the blood, generate adenosine triphosphate (ATP) in the mitochondria, and contract with force sufficient to support the intended activity.

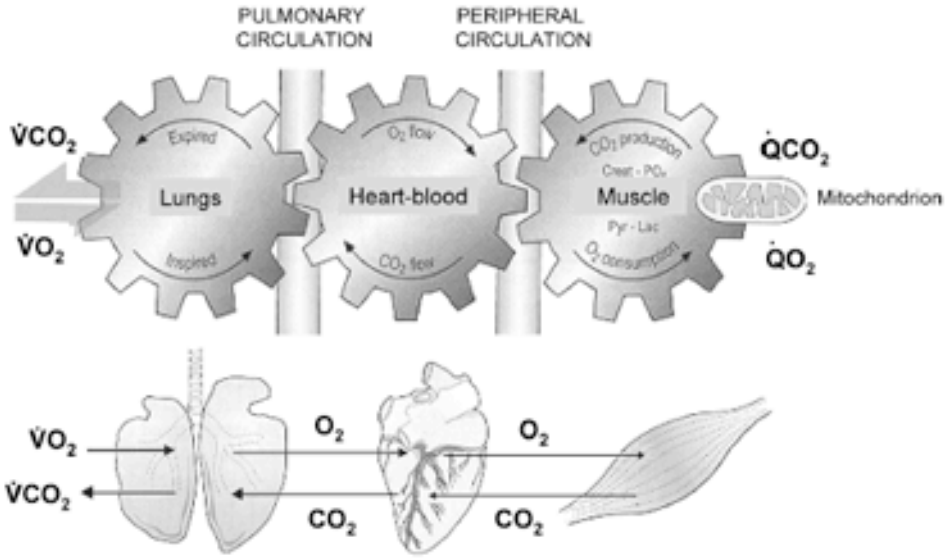

Figure 8. The pathway for oxygen (from Exercise and Sport Science Review 2007)

The systems do not work independently but rather in a highly coordinated manner. The most significant interdependence is the delivery of oxygen to the working muscles. The lungs must efficiently oxygenate blood returning from the venous system, and the left heart must then distribute this oxygenated blood to skeletal, cardiac, and respiratory muscles in proportion to the amount of work being done by the individual muscles. All of this coordination must occur in proportion to the

amount of work being performed, whether it is mild, moderate, or extreme exercise. If we define $\dot{V}O_2$max as the maximal capacity of the pulmonary and cardiovascular system to take up and transport oxygen to the exercising muscles and of the exercising muscles to extract and utilize oxygen from the blood during progressive exercise, $\dot{V}O_2$max is the product of the maximal cardiac output and the maximal arteriovenous oxygen difference (Fick equation, see below). Pathology in any of the important systems noted above can lead to limitations in an individual's exercise tolerance (i.e. $\dot{V}O_2$max). In patients with cardiomyopathy, for example, delivery of oxygen to the exercising muscles is insufficient to support mitochondrial ATP generation and, as a result, muscle contraction. Similarly, in patients with severe chronic obstructive pulmonary disease (COPD), altered respiratory system mechanics impair ventilation and the patient cannot eliminate carbon dioxide (CO_2) being produced in the exercising muscles.

Patients often discount the importance of loss of exercise tolerance as a significant symptom and the clinical exams usually performed are not able to explain the source of the problem and further evaluation is necessary. One of the studies that can be used to determine the etiology of unexplained dyspnea on exertion is the cardiopulmonary exercise test (CPET). Cardiopulmonary exercise testing provides information regarding the body's response to exercise. Cardiovascular performance and ventilatory criteria are assessed during a progressive-intensity exercise stimulus to provide an integrated analysis of the physiologic responses required by the cardiovascular and respiratory systems to meet the metabolic demands of the skeletal muscle (i.e., the primary consumers of oxygen during exercise).This test requires 20–30 minutes to perform using either a treadmill or a cycle ergometer, during which time the patient's heart rate, oxygen saturation, and electrocardiogram (ECG) are monitored continuously, while blood pressure is measured intermittently. The individual wears a tight fitting mask to allow collection of all exhaled gases to measure minute ventilation, oxygen uptake, and carbon dioxide production. In some cases, blood gases are also measured using a radial artery catheter or intermittent arterial punctures.

Assessing Overall Exercise Capacity: Maximum Oxygen Consumption. As described above, the ability to perform sustained, vigorous exercise depends on the participation of multiple systems including the respiratory, cardiovascular, and neuromuscular systems. In particular, several important tasks must be accomplished by these and other systems to support physical activity, including: ventilation to deliver oxygen to the alveoli and eliminate carbon dioxide; gas exchange to move oxygen from the alveoli to the blood and carbon dioxide from the blood to the alveoli; maintenance of hemoglobin stores to bind and carry oxygen to the tissues; delivery of oxygenated blood to the exercising tissues and carbon dioxide to the lungs; extraction of oxygen by the muscle mitochondria where ATP is generated to support muscle contraction.

To assess an individual's capacity to perform all of these tasks and conduct sustained, vigorous exercise, one of the most useful parameters is the maximum oxygen consumption (VO_2max) a parameter which describes how much oxygen is being used by the tissues per minute. To understand the value of this parameter in assessing overall exercise capacity, we can look at the determinants of VO_2 using the Fick equation:

$$\dot{Q} = \frac{\dot{V}O_2}{CaO_2 - C\bar{v}O_2},$$

where Q indicates the cardiac output, CaO_2 indicates arterial oxygen content, and CvO_2 indicates the mixed venous oxygen content. Rearranging this equation we see that:

$$\dot{V}O_2 = \dot{Q} \times (CaO_2 - C\bar{v}O_2)$$

This tells us that oxygen consumption is a function of cardiac output and the arteriovenous oxygen content difference.

Recall that:

$$CaO_2 = [(1.39 \times Hb \times SaO_2) + (0.003 \times PaO_2)]$$

and

$$C\bar{v}O_2 = [(1.39 \times Hb \times S\bar{v}O_2) + (0.003 \times P\bar{v}O_2)]$$

where Hb = hemoglobin concentration, PaO_2= partial pressure of oxygen in arterial blood, PvO_2= partial pressure of oxygen in mixed venous blood, SaO_2= arterial oxygen saturation, and SvO_2= mixed venous oxygen saturation. Oxygen consumption is therefore dependent on the hemoglobin concentration, the arterial partial pressure and saturation of oxygen (reflecting the adequacy of the ventilatory pump and gas exchange), and the mixed venous saturation and partial pressure of oxygen (reflecting the ability of the tissues to extract and utilize oxygen). As a result, VO_2max gives us information about many of the systems that are necessary to generate sustained, vigorous exercise; the higher the VO_2max, the more effective all of these systems are at performing their tasks and the greater the person's exercise capacity. Maximum oxygen consumption will vary from individual to individual. Whereas the VO_2max for an average 30-year-old person might be 35–40 ml/kg/minute, an elite cyclist or cross-country skier might have a VO_2max of 85 ml/kg/minute. Patients with a cardiomyopathy, on the other hand, may have a VO_2max as low as 15 ml/kg/minute or less, severely limiting the capacity to perform normal activities of daily living. Maximum oxygen consumption declines with age, although that decline may be substantially delayed in physically active subjects.

Anaerobic or Ventilatory Threshold. As described before, during incremental exercise ventilation increases to deliver oxygen to the alveoli and eliminate carbon dioxide. The ventilatory threshold

(VT) is the point in which the linear increase of ventilation in relation to workload changes its slope. The VT is related to the point at which anaerobic metabolism increases in exercising muscles to sustain work when aerobic metabolic capacity can no longer meet the physiologic demands, and the body shifts to anaerobic metabolism as an additional source of energy. The VT is a noninvasive, reliable, and reproducible diagnostic/prognostic marker that is based on ventilatory dynamics as exercise intensity progresses. The VT usually occurs at approximately 45% to 65% of peak VO_2 in healthy untrained subjects, and at a relatively lower percentage of peak VO_2 among subjects with a reduced exercise capacity. A key utility of VT is that it provides information at a submaximal level of exercise intensity (i.e., it does not require a physiologically maximal exercise effort). Thus, the VT is useful as a parameter on which to base an exercise prescription for patients with a reduced maximal exercise performance (i.e. exercise intolerance).

VO2/Work Rate Relationship. In general, there is a linear relationship between increasing VO2 and the work rate (watts) achieved. The slope of this relationship reflects the ability of exercising muscle to extract O2 and to aerobically generate ATP. In general, a reduction (10 mL/min/w) throughout the exercise test or an acute flattening at a given point during exercise in the ΔVO2/ΔWR relationship suggests the possibility of a problem in O_2 transport ([Wasserman K, Hansen JE, Sue DY, et al. Normal Values. In: Weinberg R, ed. Principles of Exercise Testing and Interpretation. Philadelphia, PA: Lippincott, Williams and Wilkins, 2005:160–182]). General reductions may be seen in heart and lung disease, and disease in peripheral arterial function and/or mitochondrial myopathy, in which there are alterations in the cellular pathways involved in O2 utilization. Furthermore, a pattern of initial rise of the ΔVO2/ΔWR during exercise followed by abrupt flattening may reflect the onset of ischemia-induced left ventricular (LV) dysfunction in patients with coronary heart disease (CHD). Because consistent and accurate quantification of workload during treadmill testing is complicated by underlying variability during treadmill protocols (eg, body weight and handrail holding), assessment of the ΔVO2/ΔWR relationship is typically confined to exercise tests using a cycle ergometer.

NEAR-INFRARED-SPECTROSCOPY

Starting with the pioneering work of Jobsis over 25 years ago (1977), noninvasive near-infrared (NIR) spectroscopy (NIRS) has been used first to investigate experimentally and clinically brain oxygenation, and later muscle oxidative metabolism in pathophysiology. The physical principles of NIRS is based on the absorption of light NIR by hemoglobin (Hb) in small arterioles, capillaries and venules. Briefly, NIR light (700–1000 nm) penetrates skin, subcutaneous fat/skull, and underlying muscle/brain, and is either absorbed or scattered within the tissue (Fig. 9).

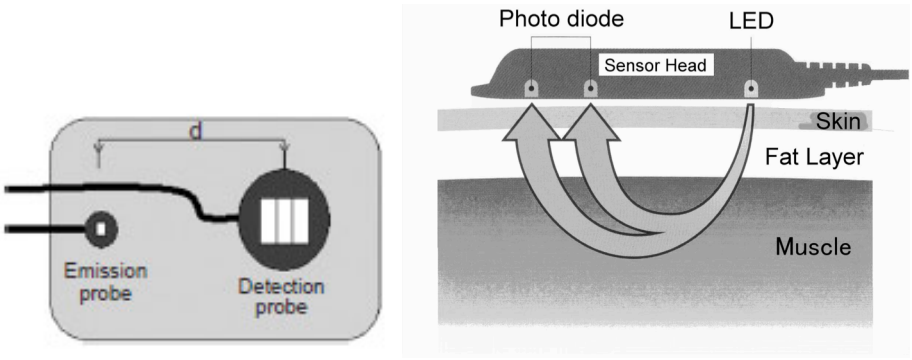

Figure 9. Schematic representation of NIR light traveling through the muscle

Based on the Beer-Lambert Law, photons migrate successfully through tissue regions with the least absorbance. In the smallest vessels, minimal light absorption allows for multiple complete passage of photons along their path through tissue and therefore changes in chromophore concentrations can be detected from the light absorption. In contrast, light emitted into larger vessels (arteries and veins) is almost completely absorbed since the molar quantity of blood is so comparatively large. The relatively high attenuation of NIR light in tissue is due to: (a) O_2-dependent absorption from chromophores of variable concentration, i.e., hemoglobin (Hb), myoglobin (Mb) (in the muscle only), and cytochrome oxidase; (b) absorption from chromophores of fixed concentration (skin melanine); or (c) light scattering. Thus, the oxygen saturation of the investigated tissue can be estimated. The parameters commonly derived from NIRS studies in humans are: deoxygenated Hb and myoglobin (Mb) concentrations, oxygenated Hb and Mb concentrations, and the sum of the two variables which is related to changes in the total Hb volume in the muscle region of interest. Moreover, the ratio of oxyhemoglobin (HbO2) to total hemoglobin (THb) can be calculated to estimate an index of changes in tissue O2 saturation relative to rest. This parameter indicates the balance between O2 delivery and tissue O2 consumption. The brain/muscle volume measured by the different NIRS approaches is still controversial. However, it is generally accepted that, for a source-detector separation of 3 cm, the region of maximum brain/muscle sensitivity will be found between the source and detector fiber tip location, and roughly 1.5 cm below the surface of the skin, though a banana-shaped region of sensitivity extends both above and below this depth (Strangman et al., 2002a).

Several types of NIRS spectrophotometry devices have been developed varying in sophistication and ease of application, algorithms used and number of wavelengths employed ([Ferrari M, Binzoni T, Quaresima V. Oxidative metabolism in muscle. Phil Trans R Soc Lond B 1997: 352: 677–683]). The most versatile and widely used devices are continuous wave spectrometers which do not allow quantitative measures of absolute concentrations of the chromophores, but instead provide concentration changes deviating from a baseline value (i.e. rest) during variations in O_2 availability and utilization.

Today, NIRS is extensively utilized to study circulatory and muscle metabolic pathologies in several situations. Distinct abnormalities in tissue oxygenation have been detected with NIRS in patients with peripheral vascular disease (McCully KK, Halber C, Posner JD. Exercise-induced changes in oxygen saturation in the calf muscles of elderly subjects with peripheral vascular disease. J Gerontol 1994: 49: B128–B134). Pronounced muscle O_2 desaturation during exercise and delayed recovery after exercise have been observed in heart failure patients with impaired cardiac output and muscle blood flow (Hanada A, Okita K, Yonezawa K, Ohtsubo M, Kohya T, Murakami T, Nishijima H, Tamura M, Kitabatake A. Dissociation between muscle metabolism and oxygen kinetics during recovery from exercise in patients with chronic heart failure. Heart 2000: 83:161–166). Furthermore, abnormalities in muscle oxidative metabolism in individuals with mitochondrial myopathies (Grassi B, Marzorati M, Lanfranconi F, Ferri A, Longaretti M, Stucchi A, Vago P, Marconi C, Morandi L. Impaired oxygen extraction in metabolic myopathies: detection and quantification by near-infrared spectroscopy. Muscle Nerve 35: 510–520, 2007) have been detected with NIRS. These observations suggest that NIRS may have a diagnostic role in assessing the presence and extent of both circulatory and metabolic disorders.

VO_2 KINETICS

The study of VO_2 kinetics starts in the nineteenth-century thanks to the English Nobel laureate, Archibald Vivian Hill, who studied the dynamics of metabolic processes in muscles isolated from small animals and used these data to develop his ideas about energetics within human athletes. The term ‘kinetics’ indicates ‘the study of the action of force in producing or changing something’ and, therefore, the term “VO_2 kinetics” is utilized to identify the study of the physiological mechanisms responsible for the dynamic VO_2 response to exercise and its subsequent recovery.

Other than when sleeping or being completely immobile, humans are rarely in a metabolic steady state. At the onset of movement or dynamic exercise such as cycling or running, the energetic requirements of the contracting muscles increase immediately with the first contraction in what has been termed a ‘square-wave’ fashion. However, as demonstrated in Figure 10, neither the increase in pulmonary nor muscle VO_2 have ‘square-wave’ response profiles.

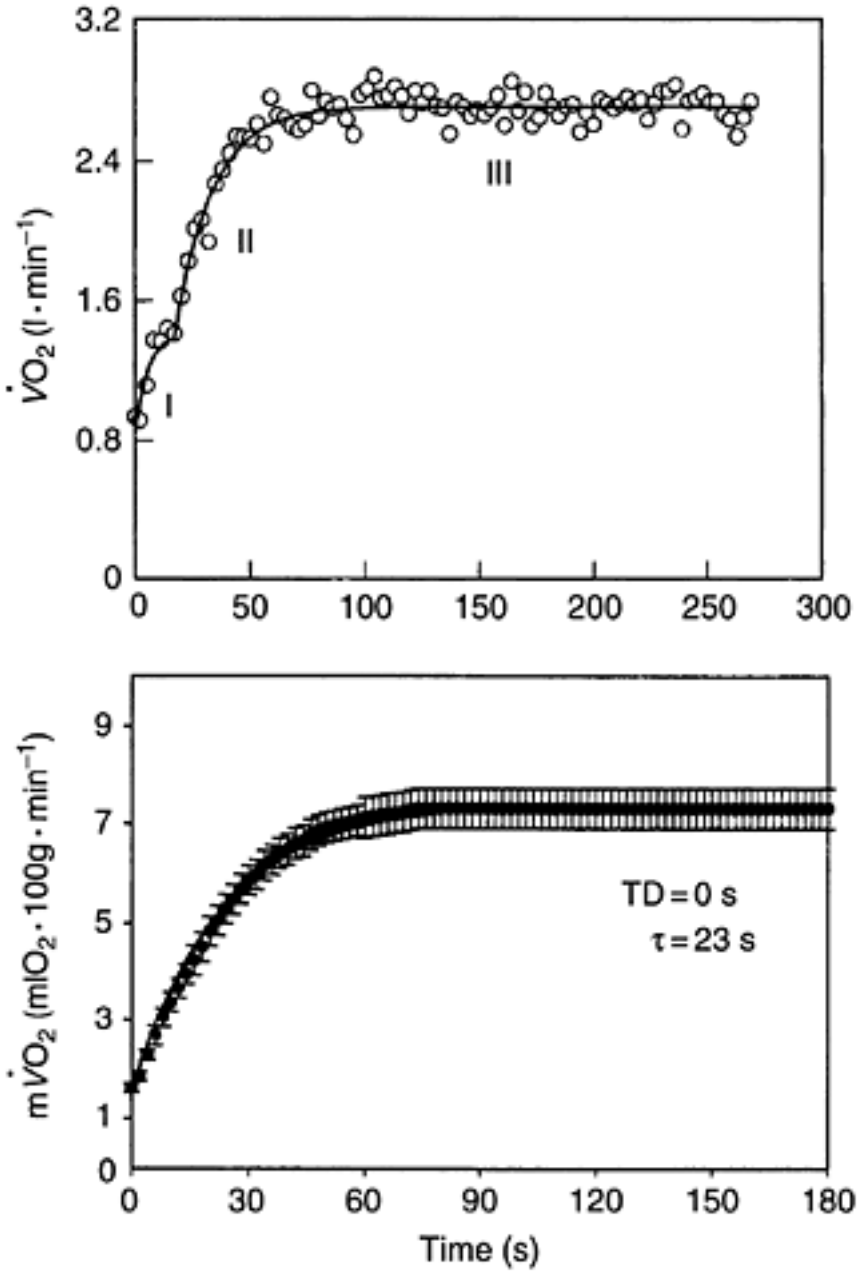

Figure 10. *Upper:* Actual breath-by-breath alveolar VO_2 response across the transient from unloaded cycling (0W) to a moderate work rate for a representative subject (data from Grassi *et al.,* 1996). *Lower:* Increase in muscle VO_2 at the onset of muscle contractions (time 0) in rat spinotrapezius measured directly from capillary red blood cell flux and microvascular O2 pressures (Behnke *et al.,* 2002).

The response demonstrates considerable inertia and, depending on the health or fitness of the individual and the exercise intensity, may take from 2 to 15 or more min to achieve the steady-state values (Figures 1.8 and 1.9). More specifically, at the onset of constant workrate exercise, there is an early rapid increaseof VO_2 that is typically initiated within the first breath (Phase I, Fig. 10). This initial increase in Phase I is followed by a rapid exponential increase in (Phase II) with a time constant or τ of some 20–45s (healthy individuals) that drives to the actual, or towards the initially anticipated, steady-state value (Phase III) within 3 minutes. Phase I represents the O2 exchange

associated with the initial elevation of cardiac output and thus pulmonary blood flow. Phase II reflects the arrival at the lung of venous blood draining the exercising muscles. The pulmonary kinetics in Phase II therefore largely reflect the kinetics of O2 consumption in the exercising muscles, although there is a temporal lag between events at the muscle and those recorded at the lung. For moderate intensity exercise, the onset of Phase III corresponds to the point at which cardiac output plateaus and venous O2 content reaches its nadir. At higher exercise intensities, the attainment of a steady state might be delayed or absent (Fig. 11).

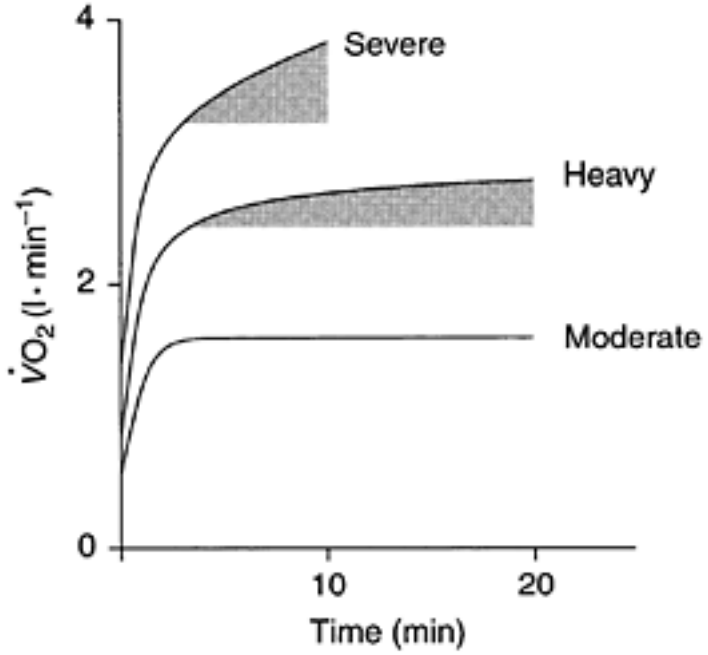

Figure 11. Schematic representation of the VO_2 response to constant work-rate exercise in the moderate (below lactate threshold), heavy (above the lactate threshold) and severe (above the asymptote of the power-time relationship for high-intensity exercise, critical power) exercise domains.

In the transition from rest or unloaded exercise to a work rate with a requirement below the anaerobic threshold, the vertical distance between the actual at a given moment and that required in the steady state represents the energy requirement that must be met from energy stores within the muscle. These stores consist principally of energy released through phosphocreatine hydrolysis and anaerobic glycolysis, with a small contribution from O2 stores (myoglobin, venous blood). The total O2 equivalent of that amount of energy is termed the O2 deficit. Thus, for a given VO_2 the faster the response (smaller τ), the smaller is the O2 deficit that will be incurred. In contrast, extremely unfit or unhealthy individuals will have a very slow response (larger τ) and will incur a high O2 deficit and thus a greater degree of intracellular perturbation (increased lactic acid, decreased PCr). Slow kinetics mandate a greater depletion of intramuscular [PCr] and a greater rate of glycogenolysis leading to greater accumulation of lactate and protons and a greater utilization of

the finite intramuscular glycogen reserves, all factors which predispose to a reduced exercise tolerance.

Model characterization of kinetics. It has been established that the VO_2 response to constant workrate exercise in Phase II is essentially exponential. This exponential nature of the response can be described with the following equation:

$$\dot{V}O_2\,(t) = \dot{V}O_2\,(b) + A\,(1-e^{-(t-TD)/\tau})$$

where (t) is the at any point in time, (b) is the baseline before the commencement of the step transition to a higher work rate, A is the steady-state amplitude of the VO_2 response, and $(1–e^{-(t−TD)/\tau})$ is the exponential function describing the rate at which VO_2 is rising towards the steady-state amplitude. In this exponential function, t is time, TD is the time delay before the start of the exponential term and τ is the time constant, i.e. the time required for the attainment of 63% of the total amplitude. To note, the exponential increase of pulmonary VO_2 following the onset of muscular exercise is essentially a 'mirror image' of the exponential reduction of intramuscular [PCr], once the 'muscle to mouth' transport delay time has been accounted for (Rossiter, H.B., Ward, S.A., Doyle, V.L., Howe, F.A., Griffiths, J.R. and Whipp, B.J. (1999). Inferences from pulmonary O2 uptake with respect to intramuscular [phosphocreatine] kinetics during moderate exercise in humans. Journal of Physiology, 518). This strongly suggests that muscle kinetics are principally under feedback control from one or more of the products of high-energy phosphate splitting.

It is important to remember that, in the non-steady state, the relationship between muscle and pulmonary is distorted by at least three factors: the transit delay between the muscle and the lung; muscle and venous O2 stores; and the kinetics of cardiac output (a given a-vO2 difference at the muscle will be associated with a faster blood flow when it reaches the lung 5–20s later). However, direct experimental measurements confirm that the Phase II pulmonary kinetics provide a close approximation of the kinetics (amplitude and time constant) of increased muscle O2 consumption following the onset of exercise (Grassi, B., Poole, D.C., Richardson, R.S., Knight, D.R., Erickson, B.K. and Wagner, P.D. (1996). Muscle O2 uptake kinetics in humans: implications for metabolic control. Journal of Applied Physiology, 80). For heavy and severe intensity exercise, an additional exponential term is required for satisfactory fitting of the on-response following the completion of Phase I:

$$\dot{V}O_2\,(t) = \dot{V}O_2(b) + A_p\,(1-e^{-(tp-TDp)/\tau p}) + A_s\,(1-e^{-(ts-TDs/\tau s})$$

where Ap and As are the amplitudes of the primary and slow components, respectively, TDp and TDS are the independent time delays before the commencement of the primary and slow components, respectively, and τp and τs are the time constants for the primary and slow components, respectively. It has been demonstrated that the kinetics in heavy and severe exercise are better fit with two exponential terms than with a single exponential term following the

completion of Phase I. This suggests that the slow component does not start at exercise onset but 'appear' later in exercise. The time at which the slow component begins to develop depends on the exercise intensity (it begins earlier at higher work rates within the heavy and severe domains) but is normally at ~2min following the onset of exercise (Burnley, M., Jones, A.M., Carter, H. and Doust, J.H. (2000). Effects of prior heavy exercise on phase II pulmonary oxygen uptake kinetics during heavy exercise. Journal of Applied Physiology, 89, 1387–96).

Med. Sci. Sports Exerc., Vol. 44, No. 5, pp. 771–775, 2012.

Improved Exercise Tolerance after Enzyme Replacement Therapy in Pompe Disease

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Running title: Exercise tolerance in Pompe's disease

ABSTRACT

Purpose: Enzyme replacement therapy (ERT) has recently became available for Pompe's disease. Data on the effects of ERT on physiological variables related to exercise tolerance have never been published. **Methods:** Pulmonary gas exchange, cardiac output (by impedance cardiography), *vastus lateralis* muscle O_2 extraction (by near-infrared spectroscopy) were determined during cycle ergometer exercise in a 50-year-old patient BEFORE and after 1, 12 and 24 months of ERT. **Results**: At the same constant-workload submaximal exercise, rates of self-perceived exertion and values of respiratory exchange ratio, pulmonary ventilation and heart rate were lower during ERT *vs*. BEFORE, suggesting an increased exercise tolerance. Peak oxygen uptake ($\dot{V}O_2$peak) increased by ~35% from BEFORE (0.64 L/min, or 11.4 ml/kg/min) to 1 month (0.88 L/min, or 15.7 ml/kg/min) of treatment, and did not significantly change thereafter. Also peak cardiac output significantly increased during ERT, whereas peak skeletal muscle fractional O_2 extraction was unchanged compared to BEFORE. **Conclusions**: Improvements of peak exercise capacity and exercise tolerance at submaximal workloads were observed in a patient with Pompe's disease after 1 month of ERT, with no further changes during the ensuing treatment period (up to 24 months).

Keywords: glycogen storage disease type II; cycle ergometer exercise; cardiac output; O_2 uptake; near-infrared spectroscopy.

DISCLOSURE OF FUNDING: This study was financially supported by Telethon-UILDM (Italy), Project GUP08007

INTRODUCTION

Paragraph number 1. Pompe's disease, also known as glycogenosis type II or acid maltase deficiency, is an inherited myopathy caused by the deficiency of lysosomal acid a-glucosidase (GAA) or acid maltase. The enzyme catalyzes the hydrolysis of a-1,4 and a-1,6 links of glycogen and its deficiency leads to intra-lysosomal accumulation of glycogen. The disease presents as a continuum of clinical phenotype, varying according to age at onset, organ involvement and severity of progression. The severe infantile disease is characterized by generalized muscle weakness, hypertrophic cardiomyopathy and an invariably fatal outcome by 1 year of age. Patients with late-onset Pompe's disease present with progressive muscle weakness that can also affect pulmonary function. A considerable number of patients become wheelchair dependent and may require assisted ventilation later in life (8). An inverse correlation is usually observed between the amount of residual GAA activity and disease severity, and in general the symptoms do not emerge until the GAA activity remains above 30% of average normal activity (23). According to Nascimbene et al. (12) patients with Pompe's disease would have a predominant expression of inactive forms of acid a-glucosidase protein and severely reduced or absent levels of mature forms, and disease severity would be primarily determined by the amount of functional protein.

Paragraph number 2. Until recently, no effective therapy for Pompe's disease was available, even if positive effects have been reported with a combination of high-protein and low-carbohydrate nutrition and exercise (18). Enzyme replacement therapy (ERT) with recombinant human a-glucosidase became available in 2000 (20), and currently a number of studies have been published on the efficacy and safety of ERT in Pompe's disease. Clinical studies in infants have shown that ERT led to improvement in skeletal and cardiac muscle function and to increased survival in many patients (10). As far as the effects of this treatment in older children and adults is concerned, data were available only for a limited number of patients (2,16,19,25). A few months ago the first randomized double-bind, placebo-controlled trial on 90 patients with late-onset Pompe's disease was published (22). Treatment with a-glucosidase was associated with improved walking distance and stabilization of pulmonary function over an 18-month period. To our knowledge, data on the effects of this therapy on physiological variables related to exercise tolerance have never been published. This would be relevant, since in patients with Pompe's disease the reduced exercise tolerance significantly affects the patients' clinical picture and quality of life.

Paragraph number 3. We report a 24-month follow-up of an adult patient who underwent ERT. In particular, data related to cardiovascular and metabolic responses to exercise and respiratory

function are provided. All measurements were non-invasive, so they could be easily repeated as a function of time.

PATIENT AND METHODS

Paragraph number 4. The patient, a 50 year-old female **(**height 162 cm, body mass 57 kg and body mass index 21.7 $kg \cdot m^{-2}$), was first investigated at the age of 38 years because of muscle weakness and easy fatigability. Muscle biopsy revealed few myopathic changes and PAS-positive and phosphatase-positive intracytoplasmic vacuoles. Pompe's disease was confirmed by the observation of a reduced GAA activity in muscle tissue. During the follow-up period, a slowly progressive decline in muscle strength, mainly involving pelvic girdle and proximal lower limb muscles was observed. At the age of 48 years, the patient was still able to walk and work but complained of difficulty in climbing stairs and standing up from a chair and also experienced feeding and swallowing difficulties. A clinically significant cardiac involvement was excluded by electrocardiogram and echocardiogram. ERT with recombinant human a-glucosidase (Myozyme^{TM}, Genzyme Corporation, Cambridge, Mass) was administered intravenously at a dose of 20 mg/kg every two weeks. Before treatment (BEFORE) and after 1, 12 and 24 months of ERT the patient was evaluated in our laboratory. The subject was fully informed of any risk and discomfort associated with the experiments before giving her written informed consent to participate to the study, which was approved by the local institutional ethics committees. All procedures were in accordance with the recommendations found in Declaration of Helsinki (2000) of the World Medical Association.

Paragraph number 5. Assessment included evaluations of neuromuscular, pulmonary functions and exercise tolerance. Muscle strength was evaluated by manual muscle testing and graded according to the 5 point score Medical Research Council (MRC) scale (11). The following muscles were bilaterally tested: biceps, triceps, deltoid, digit flexors, arm intra-extrarotators, ilio-psoas, quadriceps, thigh abductors, adductors, flexors, and extensors, anterior and posterior tibialis, head flexors and extensors. The best side was considered. Total score ranged from 0 ("total paralysis") to 75 ("normal strength"). A Functional evaluation by the Walton scale (5) was also performed. Five functions were analyzed: gait (score range 1-7), climbing stairs (score range 1-7), getting up from a chair (score range 1-6), standing from the floor (score range 1-7), rising arms above the head (score range 1-6). Score 1 indicated a normally performed function, 6 or 7 impossibility to perform function.

Paragraph number 6. Since late-onset patients may present asymptomatic diaphragmatic weakness, revealed as a difference between pulmonary function variables between sitting and supine positions greater than 20% ("postural drop") (21), forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) were measured both in sitting and supine position.

Paragraph number 7. Exercise tolerance was assessed on an electronically braked cycle ergometer (Corival, Lode, The Netherlands). After sitting for a few minutes at rest the patient performed at 15 watts (W) for 6 minutes, thereafter the workload was increased by 10 W and the patient exercised for other 6 minutes or until voluntary exhaustion (defined by the inability to maintain the imposed pedalling frequency of 60 revolutions/min). At 12 and 24 months, after the exercise protocol described above and 20 minutes of recovery, the patient was able to perform an incremental exercise to voluntary exhaustion: 5 W/min increments were given after an initial step of 4 min at 15 W. Levels of self-perceived exertion (RPE) were assessed using the validated Borg scale (1). For cardiovascular, gas exchange, and muscle oxygenation variables mean values were calculated during the last 30 seconds of each load. The highest values reached at exhaustion by the patient, during either the constant workload or the incremental exercise, were taken as "peak" values.

Paragraph number 8. Pulmonary ventilation ($\dot{V}E$, expressed in BTPS), O_2 uptake ($\dot{V}O_2$), and CO_2 output ($\dot{V}CO_2$), both expressed in STPD, were determined breath-by-breath by a metabolic cart (SensorMedics Vmax29c; The Netherlands). Arterial blood oxygen saturation (SaO_2) was continuously monitored by pulse oximetry (Biox 3740, Pulse Oximeter, Ohmeda, Denver, CO) at the ear lobe. Heart rate was monitored from the electrocardiogram; stroke volume was estimated beat-by-beat by impedance cardiography (Physio Flow, Manatec, Paris, France). The accuracy of this device has been previously evaluated during incremental exercise in healthy subjects against the direct Fick method (15); in that study the correlation coefficient between the two methods was r = 0.946 ($P<0.01$), the mean difference was equal to -2.78 ± 12.33 (2 SD) %, and the accuracy of the impedance cardiography method was recognized to be "acceptable". Cardiac output (CO) was calculated as HR·SV. The Fick equation was utilized to calculate systemic arteriovenous O_2 difference ($D_{a-}\bar{\nu}O_2$). Oxygenation changes in vastus lateralis muscle were evaluated by near-infrared spectroscopy (NIRS). A portable NIR single-distance continuous-wave near-infrared spectroscopy (HEO–100, Omron, Japan), was used. Concentration changes of oxygenated Hb+Mb (Δ[oxy(Hb+Mb)]) and deoxygenated Hb+Mb (Δ[deoxy(Hb+Mb)]), with respect to an initial value arbitrarily set equal to zero during the resting condition preceding the test, were calculated and expressed in arbitrary units (17). (Δ[deoxy(Hb+Mb)]) was taken as an estimate of skeletal muscle O_2 extraction because this variable, unlike (Δ[oxy(Hb+Mb)]), is relatively insensitive to changes in

blood volume (3,6). A "physiological calibration" of the Δ[deoxy(Hb+Mb)] values was performed during a transient limb ischemia: data obtained during exercise were expressed as a percentage of the values determined by obtaining a maximal deoxygenation of muscle, after the exercise period, by pressure cuff inflation (at 300-350 mm Hg) carried out at the inguinal crease of the thigh for a few minutes until Δ[deoxy(Hb+Mb)] increase reached a plateau. Further details on the methods can be found in a previous paper by our group (7).

RESULTS AND DISCUSSION

Paragraph number 9. No side effects of therapy were observed. The patient reported a progressive subjective improvement of daily performance, and particularly less fatigability both at work and during the housekeeping activities. After 9 months of treatment she spontaneously started to perform 2-3 times per week short sessions (about 15 minutes) of exercise on a stationary bicycle.

Paragraph number 10. Muscle strength total score varied from 57 before therapy to 60 after 12 months and was unchanged at 24 months. A slight improvement was observed also in the Walton score, that changed from 20 (BEFORE) to 18 and 16 (after 12 and 24 months, respectively).

Paragraph number 11. Before the start of treatment FVC was 95 and 72% of the predicted normal in sitting and supine position, respectively. FVC showed an improvement during ERT whereas FEV_1 remained stable throughout the treatment period (see Figure 1). In individuals without restrictive respiratory impairment differences in pulmonary function variables from sitting to the supine positions usually vary by no more than 10% (13). Patients with late-onset Pompe's disease, due to presence of marked diaphragmatic weakness, may present a "postural drop" (or an amount of volume lost between these positions greater than 20%). In our patient this feature was evident BEFORE and persisted during the 24 months of treatment.

Paragraph number 12. The patient tolerated the exercise tasks relatively well and she never reported muscle cramps. ERT increased peak exercise capacity and exercise tolerance at submaximal workloads. The positive effects of ERT on exercise tolerance at submaximal workloads are highlighted by the data obtained during the constant workload exercise at 15 W (see Table 1). During ERT the patient referred progressively lower levels of self-perceived exertion, and achieved lower values of respiratory exchange ratio, pulmonary ventilation and heart rate. All these changes are considered, for the same absolute workload, classic signs of improved exercise tolerance. At BEFORE the patient was able to perform at 25 W for only 30 seconds, whereas at 12-month she completed the 6-min 25-W load.

Paragraph number 13. Although the incremental test could not be carried out in BEFORE, the "peak" values obtained during the incremental protocol after 12 and 24 months of ERT should be comparable to those obtained at exhaustion during the constant workrate tests. It is indeed well accepted that the values obtained at exhaustion during a constant load exercise lasting a few minutes are pretty similar to the values obtained at exhaustion during an incremental exercise (9); being determined at exhaustion, both values can be defined "peak" values of aerobic function. Peak values of $\dot{V}O_2$, CO and $D_{a-}\bar{\nu}O_2$ are shown in the Figure 2. $\dot{V}O_2$peak increased by ~35% from BEFORE (0.64 L/min, or 11.4 ml/kg/min) to 1 month (0.88 L/min, or 15.7 ml/kg/min) of ERT, and did not significantly change thereafter. $\dot{V}O_2$peak values obtained during ERT correspond to ~4 METs, or 4 times the resting energy expenditure (1 MET = 3.5 $mlO_2 \cdot kg^{-1} \cdot min^{-1}$), and to about 57% of the sex- and age-predicted peak value. The increase in $\dot{V}O_2$peak was associated with a substantial increase in COpeak, that is in the peak capacity of cardiovascular O_2 delivery. COpeak increased indeed from ~10 L/min in BEFORE to ~17-18 L/min during treatment; also for this variable the increase occurred early (within the first month) during ERT , with no significant further changes during the ensuing treatment period. Both HRpeak (which increased from 130 beats/min to 147 and 148 beats/min at 1 and 24-month, respectively) and peak stroke volume (from about 77 mL at BEFORE to about 118 at 1-month and 133 mL at 12-month) contributed to the increased COpeak. Arterial blood oxygen saturation (SaO_2) did not significantly decrease during any of the exercise protocols, suggesting a substantially normal cardiopulmonary function.

Paragraph number 14. On the other hand, both the calculated systemic $D_{a-}\bar{\nu}O_2$ peak and the measured (by NIRS) peak fractional O_2 extraction by the vastus lateralis muscle were not significantly affected by ERT. The calculated peak $D_{a-}\bar{\nu}O_2$peak during exercise did not change from the resting value (about 5-6 ml O_2/100 ml), suggesting an impaired capacity to increase O_2 extraction. Also this finding was confirmed by NIRS data. In fact, during all exercise protocols, (Δ[deoxy(Hb+Mb)] values, taken as an estimate of skeletal muscle fractional O_2 extraction (for details see [7]), were substantially unchanged compared to those determined at rest.

Paragraph number 15. As mentioned above, in the patient ERT improved exercise tolerance, as shown both by submaximal and peak data. The substantial increase in peak $\dot{V}O_2$ was associated with a substantial increase in peak cardiac output, whereas peak fractional O_2 extraction by skeletal muscles was not affected. It would be an oversimplification, however, to say that the lack of increase in skeletal muscle O_2 extraction demonstrates a lack of improvement in skeletal muscle oxidative metabolism. In the presence of an increased peak O_2 delivery, indeed, peak O_2 uptake by

skeletal muscles (as estimated from pulmonary $\dot{V}O_2$) significantly increased. The specific effects of ERT (and exercise training, see below) on oxidative metabolism within skeletal muscles of patients with Pompe's disease should be evaluated by more specific investigations. Data from infants with Pompe's disease indicate that cardiac muscle responds well to ERT, whereas the response of skeletal muscle is highly variable (24). Similarly, ERT treatment in the murine knock-out model of the disease reversed the pathology in cardiac muscle but has been less effective in skeletal muscle (4,14). Unfortunately, muscle biopsies were not performed in the present study.

Paragraph number 16. As discussed above, the subjective improvement observed by the patient during the first few months of ERT induced her to spontaneously adopt a light exercise training protocol. Thus, it cannot be excluded that the beneficial effects described in the present study after the first few months of ERT can be attributed, at least in part, to the increased level of physical activity. It must be noted, however, that most of the beneficial effects (see *e.g.* those on $\dot{V}O_2$peak) were already described after 1 month of ERT, that is well before the patient spontaneously started training (which occurred after about 9 months of ERT). It can be hypothesized that ERT and exercise training could have additive positive effects on these patients' exercise tolerance and, ultimately, on their quality of life. We of course recognize that the present data were obtained only in one patient, and that they need to be confirmed in studies performed on a larger number of patients.

Acknowledgements

Financial support by Telethon-UILDM (GUP08007) is acknowledged. The authors report no conflict of interest. We thank the patient for participating in this study and Drs. Francesca Lanfranconi, Michele Belletti and Takashi Migita for excellent technical assistance. The results of the present study do not constitute endorsement by the American College of Sports medicine.

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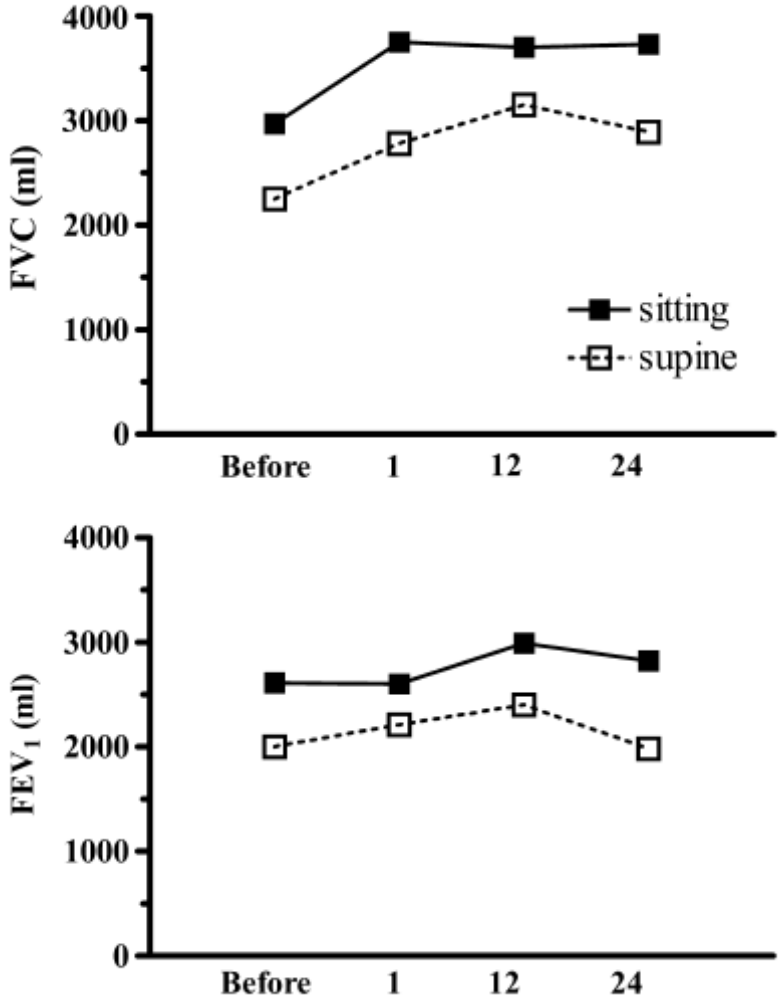

FIGURE 1. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) measured before treatment (BEFORE) and after 1, 12 and 24 months of ERT both in sitting and supine position.

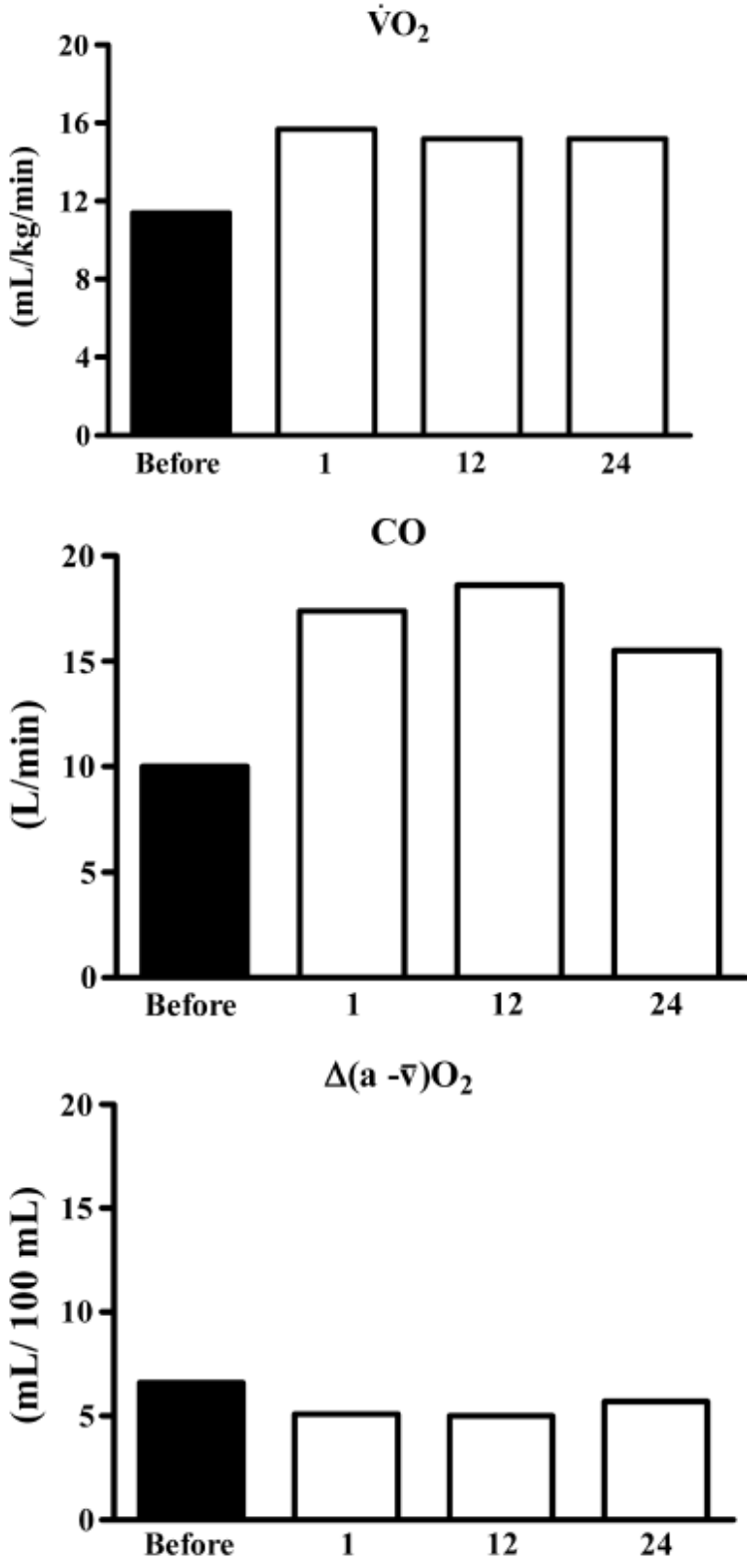

FIGURE 2. Peak values of oxygen uptake ($\dot{V}O_2$), cardiac output (CO) and systemic arteriovenous O_2 difference ($D_{a-}\bar{v}O_2$) obtained before treatment (BEFORE) and after 1, 12 and 24 months of ERT.

TABLE 1. Values obtained during the exercise at 15 W (average of the last 30 s) before and after 1, 12, and 24 months of ERT.

	R	$\dot{V}_E$ (L·min^{-1})	HR (beats·min^{-1})	RPE
BEFORE	1.16	38.4	125	17
1 month	0.94	23.3	130	13
12 months	0.85	22.9	122	10
24 months	0.94	24.5	111	10

R, gas exchange ratio; $\dot{V}_E$, pulmonary ventilation.

Neuromuscular Disorders 22 (2012) S230–S234

Exercise testing in late-onset glycogen storage disease type II patients undergoing enzyme replacement therapy

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ABSTRACT

Enzyme replacement therapy (ERT) has recently became available for patients with glycogen storage disease type II (GSDII). Previous studies have demonstrated clinical efficacy of ERT, however, data on physiological variables related to exercise tolerance are scarce. Four GSDII late-onset patients (45 ± 6 years) performed an incremental exercise on a cycle ergometer, up to voluntary exhaustion, before (BEFORE) and after 12 months of ERT (AFTER). Peak workload, oxygen uptake, heart rate, cardiac output (by impedance cardiography) and *vastus lateralis* oxygenation indices (by continuous-wave near-infrared spectroscopy, NIRS) were determined. Peak workload and oxygen uptake values significantly increased during ERT (54 ± 30 *vs.* 63 ± 31 watt, and 17.2 ± 4.4 *vs.* 19.7 ± 3.5 ml/kg/min, respectively, in BEFORE *vs.* AFTER). On the other hand, for both peak cardiac output (12.3 ± 5.3 *vs.* 14.8 ± 4.5 L/min) and the NIRS-determined peak skeletal muscle fractional O_2 extraction, expressed as a percentage of the maximal values during a transient limb ischemia (30 ± 39 % *vs.* 38 ± 28 %), the observed increases were not statistically significant. Our findings suggest that in GSDII patients ERT is associated with a mild improvement of exercise tolerance. The findings need to be validated during a longer follow-up on a larger group of patients.

Keywords: glycogen storage disease type II; cardiac output; oxidative metabolism; near-infrared spectroscopy.

INTRODUCTION

Glycogen storage disease type II (GSDII), also known as Pompe's disease or acid maltase deficiency, is a rare, autosomal recessive, progressive neuromuscular disease caused by the deficiency of lysosomal acid a-glucosidase (GAA) or acid maltase. The enzyme catalyzes the hydrolysis of a-1,4 and a-1,6 links of glycogen and its deficiency leads to intra-lysosomal accumulation of glycogen. In patients with the classic infantile form, the deposition of glycogen in the heart, skeletal, and respiratory muscles causes severe cardiomyopathy, hypotonia, and respiratory failure, typically leading to death within the first year of age. In late-onset GSDII patients glycogen deposition is confined mainly to skeletal and respiratory muscles, causing progressive limb-girdle myopathy and respiratory insufficiency. A considerable number of patients become wheelchair dependent and may require assisted ventilation later in life. An inverse correlation is usually observed between the amount of residual GAA activity and disease severity, and in general the symptoms do not emerge until the GAA activity remains above 30% of average normal activity [1].

Until recently, no effective therapy for GSDII patients was available, even if physical activity alone [2] or in parallel with an high-protein and low-carbohydrate dietary regime has been demonstrated to improve quality of life and motor function [3]. Enzyme replacement therapy (ERT) with recombinant human a-glucosidase became available in 2000 [4], and currently a number of studies have been published on the efficacy and safety of ERT in GSDII disease. Clinical studies in infants have shown that ERT led to improvement in skeletal and cardiac muscle function and to increased survival in many patients [5]. Few studies on the efficacy of this treatment in older children and adults have been published so far [6-9]. Overall these studies demonstrated that ERT is associated, over a 12-36-month period, with a stabilization of pulmonary function and with an improved exercise tolerance, as estimated by the 6-min walking test (6MWT), which has been demonstrated to be an appropriate outcome measure [10]. However, besides being intrinsically imprecise, the 6MWT does not provide specific information on the function of the different organs and systems involved in exercise, or the mechanism of exercise limitation. Insights into these issues, together with a more precise quantification of the exercise intolerance, could derive from standard cardiopulmonary exercise testing, associated with measurements of pulmonary O_2 uptake, cardiac and skeletal muscle functions carried out during incremental exercise up to voluntary exhaustion.

In the present study we report a 12-month follow-up of four late-onset GSDII patients who underwent ERT. In particular, data related to cardiovascular and metabolic responses to exercise

and respiratory function are provided. All measurements were non-invasive, so they could be easily repeated as a function of time.

MTERIALS AND METHODS

Four late-onset GSDII patients (2 males and 2 females, 45 ± 6 years) were investigated. A clinically significant cardiac involvement was excluded by electrocardiogram and echocardiogram. ERT with recombinant human a-glucosidase (Myozyme^{TM}, Genzyme Corporation, Cambridge, Mass) was administered intravenously at a dose of 20 mg/kg every two weeks. Before treatment (BEFORE) and after 12 months of ERT (AFTER) the patients were evaluated in our laboratory. The subject were fully informed of any risk and discomfort associated with the experiments before giving their written informed consent to participate to the study, which was approved by the local institutional ethics committees. All procedures were in accordance with the recommendations found in Declaration of Helsinki (2000) of the World Medical Association.

The assessment included evaluations of pulmonary function and exercise tolerance.

Pulmonary function variables, forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1), were measured with the patients in sitting position by a spirometer

Exercise tolerance was assessed on an electronically braked cycle ergometer (Corival, Lode, The Netherlands). An incremental exercise was performed: after sitting for a few minutes at rest the patient performed at 15–30 W for 5 minutes, and thereafter the workload was increased by 5–10 W (according to the subject's estimated level of physical fitness) every minute until voluntary exhaustion was reached. For cardiovascular, gas exchange, and muscle oxygenation variables mean values were calculated during the last 30 seconds of each load. The highest values reached by the patient were taken as "peak" values.

Pulmonary ventilation ($\dot{V}E$, expressed in BTPS), O_2 uptake ($\dot{V}O_2$), and CO_2 output ($\dot{V}CO_2$), both expressed in STPD, were determined breath-by-breath by a metabolic cart (SensorMedics Vmax29c; The Netherlands). Expiratory flow measurements were performed by a mass flow sensor (hot wire anemometer), calibrated before each experiment by a 3-liter syringe at three different flow rates. Tidal volume and $\dot{V}E$ were calculated by integration of the flow tracings recorded at the mouth of the subject. $\dot{V}O_2$ and $\dot{V}CO_2$ were determined by continuously monitoring partial pressures of oxygen (PO_2) and carbon dioxide (PCO_2) at the mouth throughout the respiratory cycle and from established mass balance equations, after alignment of the expiratory volume and expiratory gases tracings and A/D conversion. Calibration of O_2 and CO_2 analyzers was performed before each experiment by utilizing gas mixtures of known composition. Gas exchange ratio (R) was calculated

as $\dot{V}CO_2/\dot{V}O_2$. Heart rate (HR) was monitored from the electrocardiogram; stroke volume (SV) was estimated beat-by-beat by impedance cardiography (Physio Flow, Manatec, Paris, France). The accuracy of this device has been previously evaluated during incremental exercise in healthy subjects against the direct Fick method [11]. Cardiac output (CO) was calculated as HR·SV.

Oxygenation changes in a superficial portion of the *vastus lateralis* muscle were evaluated by near-infrared spectroscopy (NIRS). A portable NIR single-distance continuous-wave photometer (HEO–100, Omron, Japan), was used. The instrument provides separate measurements of changes in deoxygenated Hb and Mb concentrations, as well as of oxygenated Hb and Mb concentrations, expressed in arbitrary units. Further details on the method can be found in a previous article by our group [12]. For a detailed discussion regarding advantages and limitations of NIRS measurements in skeletal muscle, the reader is referred to Ferrari et al. [13]. Concentration changes of deoxygenated Hb+Mb (D[*deoxy*(Hb+Mb)]), with respect to an initial value arbitrarily set equal to zero, were calculated and expressed in arbitrary units. D[*deoxy*(Hb+Mb)] was taken as an oxygenation index, since this variable is relatively insensitive to changes in blood volume [12]. D[*deoxy*(Hb+Mb)] data were expressed as a percentage of the values determined after the exercise by obtaining a maximal deoxygenation of the muscle, by pressure cuff inflation (at about 300 mmHg) carried out at the inguinal crease of the thigh (subject in the sitting position on the cycloergometer), for a few minutes until D[*deoxy*(Hb+Mb)] increase reached a plateau.

Results were expressed as mean values ± standard deviation ($\bar{x}$ ± SD). The statistical significance of differences between means was checked by paired Students' t-test. The level of significance was set at $P<0.05$.

RESULTS

No side effects of therapy were observed. We did not use validated scales to assess quality of life, however, the patients reported a progressive subjective improvement of daily performance, and particularly less fatigability both at work and during activities of daily living. After 9 months of treatment one of the patients spontaneously started to perform 2-3 times per week short sessions (about 15 minutes) of exercise on a stationary bicycle.

One patient did not perform the spirometry tests. Data obtained in the other 3 patients are shown in Figure 1. In BEFORE FVC and FEV_1 were, on the average, 73 and 74% of the predicted normal value, respectively. For both variables, either expressed in L or as a percentage of the predicted value, ERT was associated with a slight improvement, which, however, did not reach statistical significance, presumably as a consequence of the low number of patients (see also below).

The patients tolerated the exercise testing relatively well and none of them complained of significant discomfort, pain or delayed onset muscle soreness. Peak values of work rate, $\dot{V}O_2$, CO and (Δ[deoxy(Hb+Mb)] are shown in Figure 2 (also for Δ[deoxy(Hb+Mb)] peak the data were not available for one of the patients). $\dot{V}O_2$peak increased by ~10% from BEFORE (1.01 ± 0.42 L/min, or 17.2 ± 4.4 ml/kg/min) to AFTER (1.14 ± 0.36 L/min, or 19.7 ± 3.5 ml/kg/min). On the average $\dot{V}O_2$peak values obtained in AFTER correspond to ~5 METs, or 5 times the resting energy expenditure (1 MET = 3.5 $mlO_2 \cdot kg^{-1} \cdot min^{-1}$), and to ~ 59% of the sex- and age-predicted peak value. The increase in $\dot{V}O_2$peak was associated with a clear tendency for an increase in COpeak, from 12.3 ± 5.3 L/min to 14.8 ± 4.5 L/min. Both HRpeak (which increased from 134 ± 16 beats/min to 145 ± 25 beats/min) and peak stroke volume (from 91 ± 29 mL to 101 ± 21 mL) contributed to the increased COpeak. For COpeak, HRpeak and SVpeak the increase, in AFTER vs. BEFORE, did not reach statistical significance. The same can be said for Δ[deoxy(Hb+Mb)] peak, taken as an estimate of skeletal muscle fractional O_2 extraction (for details see [12]).

DISCUSSION

The major finding of the present study is that in patients with glycogen storage disease type II enzyme replacement therapy is associated, over a 12-month period, with a stabilization of pulmonary function and with a mild improvement of exercise tolerance.

Two widely accessible and simple parameters, FVC and FEV_1, were used to monitor respiratory function. A nonsignificant trend in FVC and FEV_1 improvement was observed in all patients. These findings are consistent with those recently reported in larger ERT trials on late-onset GSDII patients treated for 12 [8], 18 [9] and 36 months [7]. The stabilization or the slight improvement of pulmonary function observed in our study, as well as in the previously mentioned ones, is in contrast to the progressive deterioration that characterizes the natural course of the disease (annual decline of ~ 2-3% in the percentage of predicted FVC [9]).

ERT increased peak exercise capacity and exercise tolerance. Significantly higher values of $\dot{V}O_2$peak AFTER were associated with (and presumably were responsible for) a significant improvement of exercise tolerance, as shown by the significantly higher peak work rate values. The increase in $\dot{V}O_2$peak was associated with an improvement of COpeak and Δ[deoxy(Hb+Mb)] peak. In two of the patients Δ[deoxy(Hb+Mb)] peak values were markedly lower than the values observed by our group in healthy subjects, but higher than those obtained on patients with mitochondrial myopathies or myophosphorylase deficiency [12]. Interestingly enough, in one patient

Δ[deoxy(Hb+Mb)] peak values were substantially unchanged compared with those determined at rest; in this patient, characterized by the lowest work rate and $\dot{V}O_2$ peak values, the variable was unaffected by ERT.

As nicely discussed by Poole et al. [14], it would be an oversimplification to interpret skeletal muscle fractional O_2 extraction simply as a result of "muscle factors". The enhanced peak fractional O_2 extraction described with training in two of our patients may indeed be the result of a combination of factors such as increased bulk blood flow and O_2 delivery; enhanced vasodilation and capillary recruitment; improved intramuscular matching of O_2 delivery and O_2 utilization; enhanced peripheral O_2 diffusion; improved endothelial function; reduced levels of inflammatory, catabolic and pro-apoptotic mediators and oxidative stress; increased mitochondrial volume density and activity of oxidative enzymes; enhanced oxidative phosphorylation, etc. All these factors, which could be at least in part related to a decreased disease-induced damage in muscle tissue, could explain (and be a result of) the increased exercise tolerance.

In late-onset GSDII patients skeletal muscle pathology is extremely heterogeneous, ranging from substantially unaffected fibers to a complete destruction of contractile machinery. The pathogenic mechanisms of muscle damage are still under debate, but autophagy is increasingly identified as a pivotal contributor to muscle destruction and mitochondrial abnormalities have been repeatedly found [15]. Regarding the response to ERT, clinical trials have indicated that ERT is effective in glycogen clearance in cardiac muscle, whereas a reversal of the damage in skeletal muscle has not always been achieved, and highly variable responses between patients should be expected [8,9]. Unfortunately, muscle biopsies were not performed in the present study, thus we have no data of the degree of muscle damage in the patients in BEFORE and in AFTER.

As discussed above, the subjective improvement observed by one of the patients during the first few months of ERT induced her to spontaneously adopt a home-based light exercise training protocol. Thus, it cannot be excluded that the beneficial effects described in the present study after ERT can be attributed, at least in part, to the increased level of physical activity. Recently, evidence has been provided that ERT and exercise training could have additive positive effects on these patients' exercise tolerance and, ultimately, on their quality of life [2].

For some of the variables determined in the present study (spirometry data, COpeak, Δ[deoxy(Hb+Mb)] peak) the observed increases did not reach statistical significance. This could be attributed to the low number of patients, which does not allow us to exclude the possibility of a type 2 error. Thus, the findings need to be validated on a larger group of patients and with a longer follow-up period.

In conclusion, our findings showed that in late-onset GSDII patients ERT is associated with a mild improvement of pulmonary function and exercise tolerance over a 12 month period. The improved exercise tolerance seems associated with improvements both in cardiovascular and in skeletal muscle functions. The findings need to be validated on a larger group of patients and with a longer follow-up period. In addition, the results highlight the role that cardiopulmonary exercise testing, with simultaneous non-invasive measurements of pulmonary O_2 uptake, cardiac output and skeletal muscle oxygenation, can play in the assessment and follow-up of late onset GSDII patients.

Acknowledgements

Financial support by Telethon-UILDM (GUP08007) is acknowledged. The authors report no conflict of interest. We thank the patients for participating in this study and Drs. Francesca Lanfranconi, Michele Belletti and Takashi Migita for excellent technical assistance.

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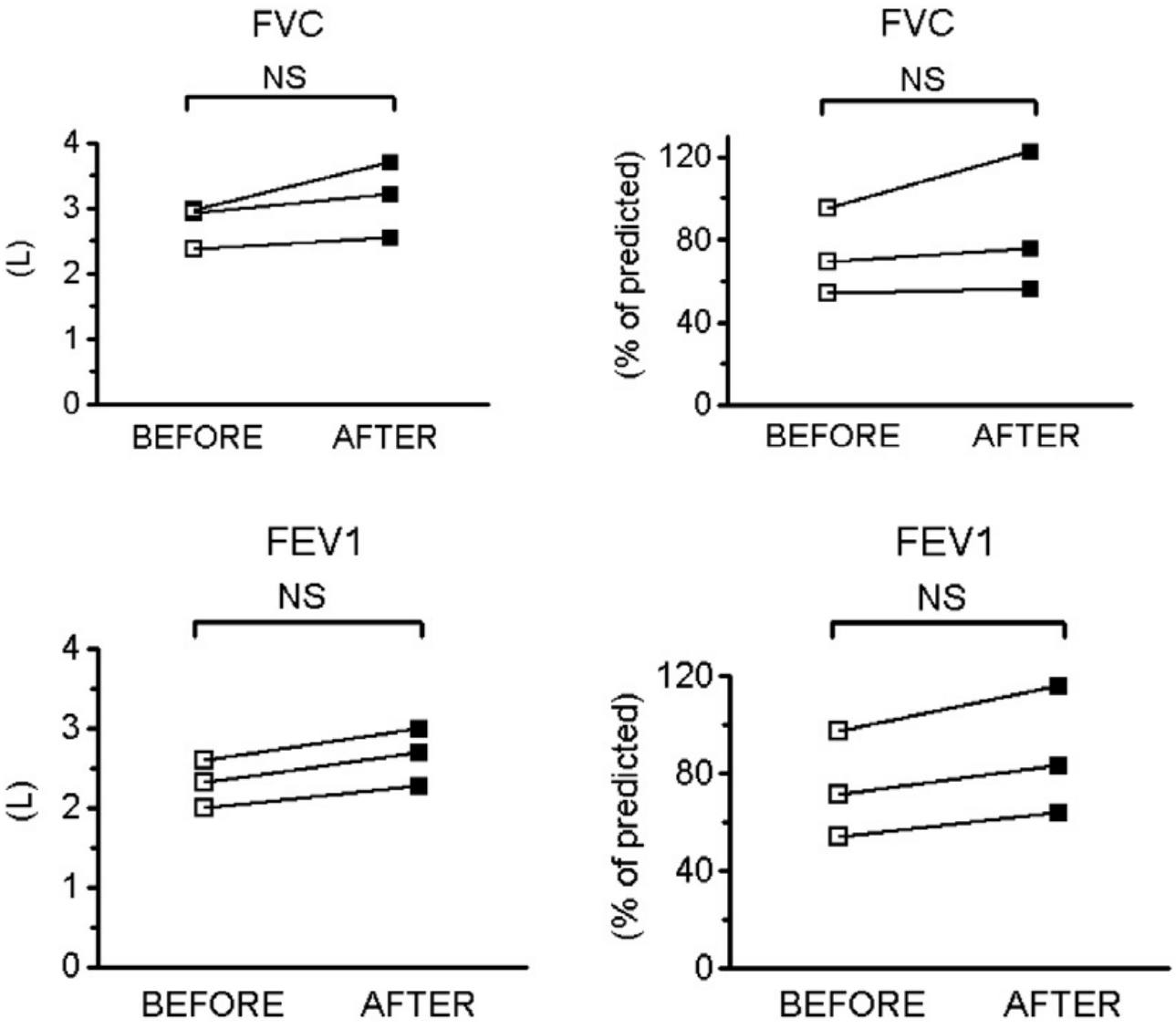

FIGURE 1. Individual values, expressed either in L or as a percentage of the predicted value, of forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) measured before treatment (BEFORE) and after 12 months of ERT (AFTER).

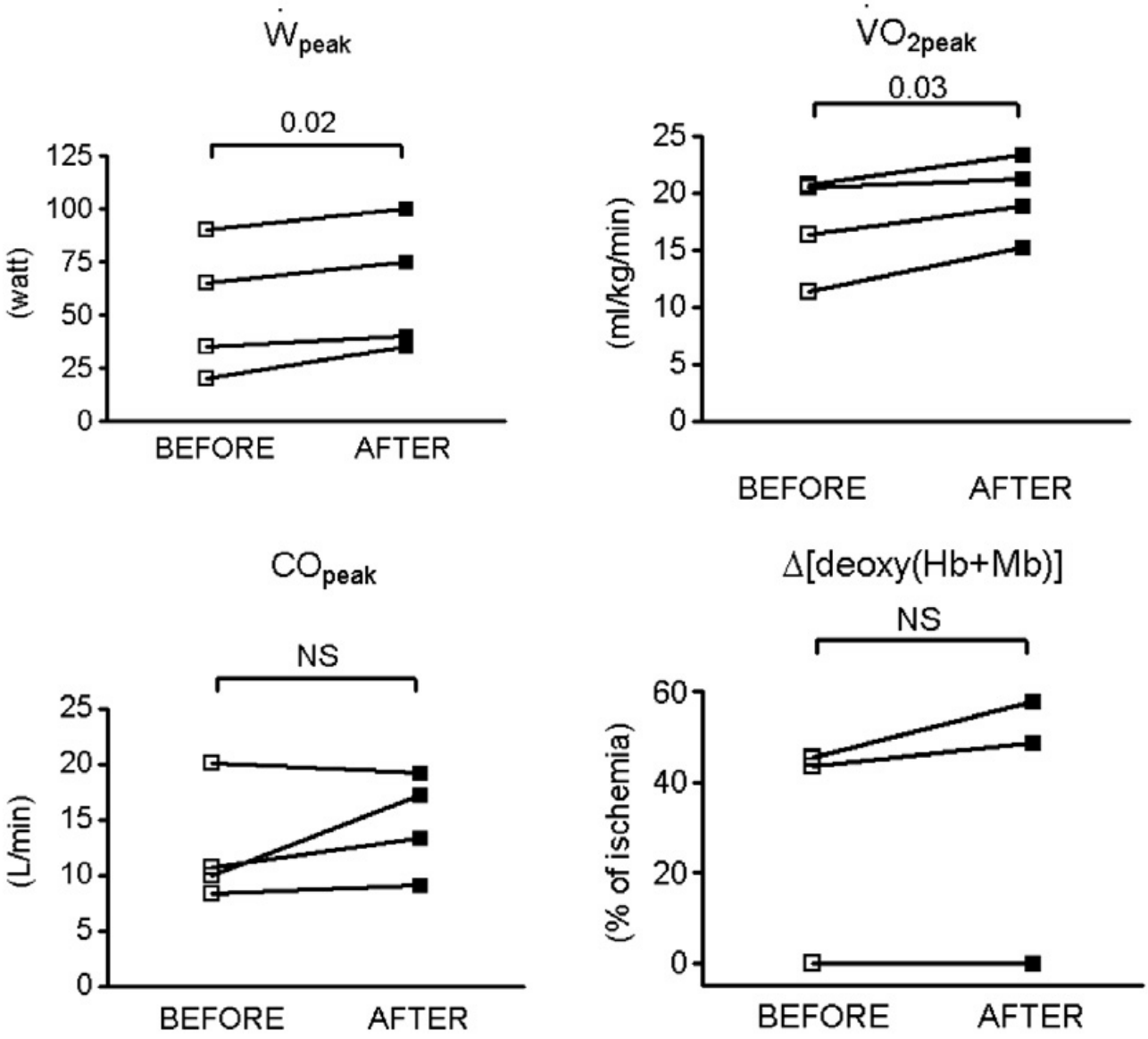

FIGURE 2. Individual peak values of work rate (W), oxygen uptake ($\dot{V}O_2$), cardiac output (CO) and Δ[deoxy(Hb+Mb)], expressed as a percentage of the maximal values during a transient limb ischemia, measured before treatment (BEFORE) and after 12 months of ERT (AFTER).

J Appl Physiol 116: 1230–1237, 2014.

The "second wind" in McArdle's disease patients during a second bout of constant work rate submaximal exercise

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RUNNING HEAD: Second wind during submaximal exercises

ABSTRACT

Patients with McArdle's disease (McA) typically show the "second wind" phenomenon, a sudden decrease in heart rate (HR) and an improved exercise tolerance occurring after a few minutes of exercise. In the present study we investigated whether in McA a first bout of exercise determines a second wind during a second bout, separated by the first by a few minutes of recovery.

Eight McA (44±4 years) and a control group of 6 mitochondrial myopathy patients (MM) (51±6 years) performed two repetitions (CWR1 and CWR2) of 6-min constant work rate exercise (~50% of peak work rate) separated by 6-min (SHORT) or 18-min (LONG) recovery. Pulmonary O_2 uptake ($\dot{V}O_2$), HR, cardiac output ($\dot{Q}$), rates of perceived exertion (RPE), vastus lateralis oxygenation (Δ[deoxy(Hb+Mb)], by near-infrared spectroscopy) were determined.

In McA $\dot{V}O_2$ (0.86±0.2 *vs.* 0.95±0.1 $L \cdot min^{-1}$), HR (113±10 *vs.* 150±13 $b \cdot min^{-1}$), $\dot{Q}$ (11.6±0.6 *vs.* 15.0±0.8 $L \cdot min^{-1}$), RPE (11±2 *vs.* 14±3) were lower, whereas Δ[deoxy(Hb+Mb)] was higher (14.7±2.3 % *vs.* -0.1±4.6 %) in CWR2-SHORT *vs.* CWR1; the "overshoot" of Δ[deoxy(Hb+Mb)] and the "slow component" of $\dot{V}O_2$ kinetics disappeared in CWR2-SHORT. No differences (*vs.* CWR1) were observed in McA during CWR2-LONG, or in MM during both CWR2-SHORT and -LONG.

A second wind phenomenon was observed in McA during the second of two consecutive 6-min constant-work rate submaximal exercises. The second wind was associated with changes of physiological variables suggesting an enhanced skeletal muscle oxidative metabolism. The second wind was not described after a longer (18-min) recovery period.

KEY WORDS: myophoshorylase deficiency; exercise tolerance; $\dot{V}O_2$ kinetics slow component; near-infrared spectroscopy.

DISCLOSURE OF FUNDING: This study was financially supported by Telethon-UILDM (Italy), Project GUP08007

INTRODUCTION

Patients with McArdle's disease (McA) are affected by an autosomal recessive muscle glycogenosis (type V) caused by mutations in the gene that encodes muscle glycogen phosphorylase. Absence of activity of this enzyme blocks the breakdown of intramuscular glycogen and significantly impairs both substrate level phosphorylation from glycolysis and oxidative phosphorylation (44-46). The impairment of oxidative metabolism results in a reduced capacity to increase muscle O_2 extraction, or arterio-venous O_2 concentration difference ($[C_{(a-v)}O_2]$), during exercise (14), and leads to a significantly lower than normal maximal (or peak) O_2 uptake ($\dot{V}O_2$) (13). McA also present an exaggerated cardiovascular response to submaximal exercise, that is, higher heart rate (HR), cardiac output ($\dot{Q}$), and muscle blood flow values, compared to healthy subjects, for the same submaximal $\dot{V}O_2$ (19, 28,29,40,44-46,49), together with a markedly slower adjustment of $\dot{V}O_2$ during constant work rate submaximal exercise (17).

A typical feature of McA is the "second wind" phenomenon (20,31). As first described by Pearson et al. (31), the second wind is characterized by the sudden decrease in HR and improvement of exercise tolerance after about 8 min of aerobic, dynamic, exercise (walking or cycling). According to Vissing and Haller (49), the second wind is pathognomonic for the disease and it is attributable to an enhanced sympatho-adrenal response and to an improved delivery of extramuscular energy substrates, free fatty acids and glucose, to working muscles, which partially compensates for the impaired glycogen breakdown (20). Other studies have demonstrated that the second wind can be induced by oral glucose (20).

No study has so far investigated if in McA a previous bout of exercise can induce a second wind phenomenon during a subsequent bout. This would be of interest also from a clinical point of view, considering that many activities of everyday life entail bouts of exercise separated by recovery periods. It could also allow patients to develop strategies (for example, having an exercise bout preceded by a "warm up" activity) which could increase their exercise tolerance. Moreover, in no previous studies the second wind phenomenon has been characterized in terms of variables intrinsically related to an enhanced skeletal muscle oxidative metabolism and to an increased exercise tolerance, such as a reduced amplitude of the "slow component" of the $\dot{V}O_2$ kinetics (23) and a reduced O_2 cost of exercise.

Also in healthy subjects a vigorous "priming" or warm-up exercise can determine a reduced amplitude of the slow component and an increased exercise tolerance during a subsequent high-intensity exercise bout (4). The mechanism(s) responsible for this phenomenon may comprise

increased muscle O_2 availability, greater muscle oxidative enzyme activity and carbon substrates supply, and altered motor unit recruitment profiles (7,18,21). Thus, at least in part (see the increased carbon substrates supply) the mechanisms potentially responsible for the "priming effect" in healthy humans could also be responsible for the second wind phenomenon in McA. It should be noted, however, that the priming phenomenon does not determine in healthy humans a lowering of HR (4), whereas lower HR (and $\dot{Q}$) are prominent effects in the second wind. In any case, in the present study the presence of a second wind phenomenon during a second bout of exercise will be evaluated also in a control group of patients affected by a mitochondrial myopathy (MM), who have similar exercise tolerance of McA (8,14), but in whom a second wind phenomenon has never been demonstrated. Thus, if the effects described during the second exercise bout would appear only in McA, this would represent strong (although indirect) evidence that they are related to a second wind phenomenon; on the other hand, if the effects would appear also in MM they would likely be related to a priming effect.

In the present study we hypothesize that in McA, but not in MM, a preceding bout of constant work rate submaximal exercise would determine, during a subsequent bout, a second wind phenomenon. Apart from the hallmark index of increased exercise tolerance, represented by lower rates of perceived exertion, more "traditional" signs of the second wind (lower HR, lower $\dot{Q}$, increased O_2 extraction) were sought after, together with other "ancillary" signs of increased exercise tolerance (lower $\dot{V}E$, lower R). In addition, we sought to determine if the second wind was associated with a decrease or with a disappearance of the slow component of the $\dot{V}O_2$ kinetics, with a lower O_2 cost of exercise, with a decrease of transient unbalances between O_2 delivery and O_2 utilization within skeletal muscles (as determined by NIRS [11,36,43]); these findings would point to an enhanced performance of skeletal muscle oxidative metabolism as one of the mechanisms of the second wind. As a secondary aim, by arbitrarily choosing a "short" (6 minutes) and a "long" (18 minutes) recovery period between exercise bouts, we also tried to get insights (also for practical purposes) into the length of the recovery period which would allow the second wind phenomenon to manifest itself. Whereas 6 minutes represent a "standard" recovery between two 6-min exercise bouts (see e.g. 4), 18 minutes were arbitrarily chosen in order to represent a longer recovery, considering that prior exercise combined with an extended recovery period [>15 min (47)] might maximize the potential for exercise tolerance to be enhanced (4).

By applying on patients methods which have been developed in the exercise physiology laboratory, the present study will follow a classic translational approach, with the ultimate aim of increasing the exercise tolerance and the quality of life of the patients.

METHODS

Subjects. Eight McA and six MM were studied. Gender distribution, age, and body mass of McA were as follows: 3 males and 5 females, age (mean ± SD) 44 ± 4 yr and body mass 75.9 ± 8.9 kg. Gender distribution, age, and body mass of MM were as follows: 5 males and 1 female, age 51 ± 6 yr and body mass 69.1 ± 7.4 kg. Patients were from the Department of Neuromuscular Diseases, Neurological Institute ‘‘Carlo Besta’’ (IRCCS), Milano. The diagnosis of McA and MM was based on clinical, morphological, biochemical, and molecular evaluations. Clinical details of the patients were similar to those reported in our previous article (14). The degree of functional impairment varied from mild (no limitations in activities of everyday life but reduced exercise tolerance) to severe (very limited exercise tolerance, impairment in activities of daily living). Exclusion criteria were the presence of neoplastic and other major neurological/psychiatric, orthopedic, rheumathologic, endocrine, pulmonary, or cardiovascular disorders. The subjects were fully informed of any risk and discomfort associated with the experiments before giving their written consent to participate to the study, which was approved by the ethics committees of the involved institutions. All procedures were in accordance with the recommendations found in the Declaration of Helsinki (2000) of the World Medical Association.

Measurements. Experiments were conducted in the morning, a few hours (at least 2 hr) after a light breakfast (about 600 kcal, 35 % fat, 55 % carbohydrate and 10 % protein). Patients were not following any specific diet. All tests were carried out under medical supervision. Subjects were monitored by 12-lead ECG.

An electromagnetically braked cycle ergometer (Corival; Lode BV, Groningen, The Netherlands) was used. Pedaling frequency was digitally displayed to the subjects. Subjects were allowed time to gain familiarity with the investigators and the experimental arrangement, were carefully instructed about the procedures, and were familiarized with the protocol using short practice runs.

On the first day the subjects performed an incremental test up to voluntary exhaustion to assess peak O_2 uptake ($\dot{V}O_2$ peak). After three minutes of unloaded pedaling, exercise was conducted at 25-50 W for 5 minutes, and thereafter the work rate was increased by 5-15 W (according to the subject’s estimated level of physical fitness) every minute. The exhaustion was defined by: (1) inability to maintain the pedaling frequency despite encouragement by the operators; (2) maximal

levels of self-perceived exertion, using the validated Borg's scale; and (3) heart rate (HR) values >85 % of the age-predicted maximum. Values of cardiovascular, ventilatory, gas exchange, and muscle oxygenation variables determined during the last 30 seconds of the exhausting load were considered "peak" values.

During the second and the third days, the patients performed two repetitions of subsequent 6-min constant work rate submaximal exercise (CWR1 and CWR2) (at a work rate corresponding to ~50 % of peak work rate); in the first case CWR1 and CWR2 were separated by a 6-min recovery period (SHORT), whereas and in the second case, (after observing at least two hours of rest) CWR1 and CWR2 were separated by a 18-min recovery period (LONG). Pedaling frequency was kept at ~60 rpm and transitions from rest to the imposed load were attained in ~3 s.

Pulmonary ventilation ($\dot{V}E$, in BTPS), O_2 consumption ($\dot{V}O_2$), and CO_2 output ($\dot{V}CO_2$), both in STPD, were determined breath-by-breath by a metabolic cart (Vmax29c; SensorMedics, Bilthoven, The Netherlands). Expiratory flow was determined by a mass flow sensor (hot wire anemometer). $\dot{V}O_2$ and $\dot{V}CO_2$ were determined by continuously monitoring PO_2 and PCO_2 at the mouth throughout the respiratory cycle and from established mass balance equations. Gas exchange ratio (R) was calculated as $\dot{V}CO_2/\dot{V}O_2$. HR was determined from the ECG signal. Stroke volume (SV) was estimated beat-by-beat by impedance cardiography (Physio Flow; Manatec, Paris, France). The accuracy of this device has been previously evaluated, in healthy subjects, during incremental exercise on a cycle ergometer, and was found to be "acceptable" (38). Cardiac output ($\dot{Q}$) was calculated as HR · SV. Systemic peak arterial-venous O_2 concentration difference ($[C_{(a-v)}O_2]$) was calculated as $\dot{V}O_2$peak/$\dot{Q}$peak. At rest and at various times (1, 3, and 5 min) during recovery, 20 μL of capillary blood was obtained from a preheated earlobe for the determination of blood lactate concentration ([La]b) by an enzymatic method (Biosen 5030; EKF, Eppendorf Italia, Milano, Italy).

Oxygenation changes in the vastus lateralis muscle were evaluated by NIRS (5,10). A portable NIR single-distance continuous-wave photometer (HEO-100; Omron, Kyoto, Japan), which adopts an algorithm based on diffusion theory (42), was utilized. The instrument provides separate measurements of changes in deoxygenated Hb and Mb concentrations, as well as of oxygenated Hb and Mb concentrations, expressed in arbitrary units. Details on the method can be found in previous studies from our group (15,27,36). Concentration changes of oxygenated Hb + Mb (Δ[oxy(Hb+Mb)]) and deoxygenated Hb + Mb (Δ[deoxy(Hb+Mb)]), with respect to an initial value arbitrarily set equal to zero, were calculated and expressed in arbitrary units. The sum of the two variables (Δ[oxy(Hb+Mb) + deoxy(Hb+Mb)]) is related to changes in the total Hb volume in the

muscle region of interest (6,12,25). Δ[deoxy(Hb+Mb)] was taken as a "deoxygenation index", because this variable is relatively insensitive to changes in blood volume (6,25). Δ[deoxy(Hb+Mb)] was considered an estimate of skeletal muscle fractional O_2 extraction, that is of the ratio between O_2 consumption and O_2 delivery (11,15). Δ[deoxy(Hb+Mb)] data were expressed as a percentage of the values determined after the exercise (at least 10 min) by obtaining a maximal deoxygenation of the muscle, by pressure cuff inflation (at about 300 mmHg) at the root of the thigh (subject in the sitting position on the cycloergometer), for a few minutes until the Δ[deoxy(Hb+Mb)] increase reached a plateau.

Kinetics analysis. $\dot{V}O_2$ kinetics were evaluated during transitions from rest to CWR. Breath-by-breath $\dot{V}O_2$ values obtained during the repetitions of the exercises were time aligned and then superimposed for each subject. Average $\dot{V}O_2$ values every 10 s were calculated. Data obtained during the first 20 s of the transition (''cardiodynamic'' phase) (37) were excluded from analysis. Thus, $\dot{V}O_2$ kinetics analysis focused on the ''phase 2'' (or ''fundamental'' component) of the response, which more closely reflects gas exchange kinetics occurring in skeletal muscles (16,34,50).

To mathematically evaluate the $\dot{V}O_2$ kinetics, data were first fitted by a monoexponential function of the type:

$$y(t) = yBAS + Af\,[1 - \exp^{-(x - TDf)/\tau f}] \qquad \text{(Eq. 1)}$$

where y*BAS* indicates the $\dot{V}O_2$ value at baseline; A*f* the amplitude of the $\dot{V}O_2$ response calculated between the baseline value and the steady-state value for the fundamental component; TD*f* is the time delay, and τ*f* the time constant of the function for the fundamental component.

To check the presence of a slow component of the kinetics (21), data were also fit by a double exponential function of the type:

$$y(t) = yBAS + Af\,[1 - \exp^{-(x - TDf)/\tau f}] + As\,[1 - \exp^{-(x - TDs)/\tau s}] \qquad \text{(Eq. 2)}$$

where A*s*, TD*s*, and τ*s* indicate, respectively, the amplitude, the time delay, and the time constant of the slow component of the kinetics.

Sometimes, after the first exponential rise, $\dot{V}O_2$ increases linearly without reaching a steady-state value. In this case, Eq. 2 does not provide a good fit of data. Thus, a third equation was also utilized, with an exponential function for the fundamental component and a linear function for the slow component (exponential + linear fitting) (38):

$$y(t) = yBAS + Af\,[1 - \exp^{-(x - TDf)/Tf}] + S\,(x\text{-}TDs) \qquad \text{(Eq. 3)}$$

where S (slope) is the angular coefficient of the linear regression of $\dot{V}O_2$ vs. time.
The equation that best fit the experimental data was determined by the F-test. That is to say, when Eq. 2 or Eq. 3 provided a better fit of the data, a slow component (46) of $\dot{V}O_2$ kinetics was present, superimposed on the fundamental component. The actual amplitude of the slow component (A's) was estimated as the difference between the average $\dot{V}O_2$ value obtained during the last 20–30 s of CWR and the asymptotic value of the fundamental component (15,38). The percentage contribution of the slow component to the total amplitude of the response (A's/Atot) was also calculated (36).

Statistical analysis. Results were expressed as mean values ± standard deviation (x ± SD). The statistical significance of differences between two means was checked by Student's t-test (two tailed, unpaired analysis). The effects of the "warm-up" exercise bout (CWR2 *vs.* CWR1) and of the group (McA *vs.* MM) on the investigated variables were checked by two-way analysis of variance (ANOVA). This analysis, however, did not yield a statistically significant difference for $\dot{V}O_2$ and $\dot{V}E$. This is likely attributable to the relatively number of patients in the two groups (McA and MM are rare diseases [see also the recent commentary by Ploutz-Snyder *J Appl Physiol* in press]). Thus, analysis of differences between CWR1 and CWR2 in MM and McA was also performed by one-way ANOVA. A Tukey's post hoc test was utilized when significant differences emerged upon ANOVA. Data fitting by linear regression or exponential functions was performed by the least squared residuals method. Comparisons between fittings with different exponential models were performed by F test. The level of significance was set at $P<0.05$. Statistical analyses were performed by a software package (Prism 5.0; GraphPad, San Diego, CA).

RESULTS

Incremental exercise. Peak values are shown in **Table 1**. Values were very similar to those obtained in McA and MM in a previous study by our group (14) and by others (19,21). $\dot{V}O_2$ peak

was ~50 % of that usually obtained in healthy age-matched subjects (30), indicating a severely reduced maximal aerobic power. HR values, significantly higher in McA than in MM, corresponded to ~96 % of the age–predicted maximum. $\dot{Q}$ peak values were only slightly lower than those usually obtained in healthy controls (39). As expected for both patients groups, $[C_{(a-v)}O_2]$ and peak skeletal muscle fractional O_2 extraction values were very low. As expected, in McA R peak values were relatively low, and [La]b peak values were not higher than those determined at rest (1.2 ± 0.1 mM). For the other variables no differences were observed between McA and MM.

Constant work rate exercises. Figure 1 shows typical examples of HR time-courses of a MM (upper panels) and of a McA (lower panels) during SHORT (left panels) and LONG (right panels). In McA, during SHORT (but not during LONG) HR values at the end of CWR2 were markedly lower (by about 50 beats · min^{-1}) than during CWR1. This second wind phenomenon is indicated by the arrow. No differences between CWR1 and CWR2 were observed in MM, either during SHORT and LONG.

Mean (± SD) values determined in the last ~30 s of CWR1 and CWR2 (SHORT and LONG recovery) are presented in **Table 2**. In McA during SHORT $\dot{V}O_2$, $\dot{V}CO_2$, $\dot{V}E$, R, HR, $\dot{Q}$ and RPE values were significantly lower in CWR2 *vs.* CWR1. On the other hand, [La]b, $[C_{(a-v)}O_2]$ and Δ[deoxy(Hb+Mb)] were significantly higher in CWR2 *vs.* CWR1. No significant differences were observed between CWR1 and CWR2 in LONG. As for MM, no significant differences were observed between CWR1 and CWR2, both in SHORT and in LONG.

$\dot{V}O_2$ and Δ[deoxy(Hb+Mb)] kinetics. Typical individual examples of $\dot{V}O_2$ kinetics of a MM (upper panels) and of a McA (lower panels) during SHORT (left panels) and LONG (right panels) are shown in **Figure 2**. As for McA, $\dot{V}O_2$ values did not reach a steady-state and a slow component was evident in CWR1. During CWR2 the slow component disappeared in SHORT, but not in LONG. This second wind phenomenon is indicated by the arrow. A slow component was not observed in MM, both during CWR1 and CWR2 (SHORT and LONG).

The parameters of $\dot{V}O_2$ kinetics are shown in **Table 3**. In both groups TD*f*, τ*f* and A*f* values were not significantly different in CWR1 *vs.* CWR2 (both in SHORT and in LONG). Gain values (G) were calculated as $\Delta\dot{V}O_2$ ($\dot{V}O_2$ at the end of CWR minus resting $\dot{V}O_2$) divided by work rate. A slow component, corresponding to ~15 % of the total amplitude of the response, was present in all McA in CWR1. In six McA the slow component was best described by a linear function (Eq. 3). In

CWR2-SHORT, but not in CWR2-LONG, the slow component disappeared. No slow component was evident in any MM. In McA A'*s*, A'*s*/A*tot* and G values were significantly lower in CWR2 *vs.* CWR1 in SHORT, but not in LONG. In MM no differences were observed for G values in CWR1 *vs.* CWR2 (both in SHORT and in LONG). In both groups of patients G values were substantially higher that those usually observed in normal subjects (~10 ml $\cdot$ min^{-1} $\cdot$ watt^{-1}), independently from the presence of the slow component.

Δ[deoxy(Hb+Mb)] kinetics are shown in **Figure 3**. In MM, in all conditions there was an initial and transient increase ("overshoot") of Δ[deoxy(Hb+Mb)] (occurring after ~45 s of exercise), which was followed by a steady state. Δ[deoxy(Hb+Mb)] values at the peak of the overshoot were significantly higher than at steady state, for both CWR1 (24.9 ± 5.1 % *vs.* 6.2 ± 3.0 %) and CWR2 (21.9 ± 4.5 % *vs.* 6.0 ± 5.4 % and 21.5 ± 5.4 % *vs.* 6.9 ± 3.5 %, respectively in SHORT and LONG). In McA values at the peak of the overshoot were higher than those at steady state during CWR1 (27.5 ± 6.0 % *vs.* -0.1 ± 4.6 %) and during CWR2-LONG (24.9 ± 6.7 % *vs*. 1.9 ± 1.0 %), whereas in CWR2-SHORT no decrease of the variable was observed after the initial increase (no overshoot was described).

DISCUSSION

We observed in McA, during the second (CWR2) of two 6-min constant work rate exercises, carried out at ~50 % of peak work rate, and separated by 6 minutes of recovery (SHORT), significant changes indicating an improved exercise tolerance and an enhanced oxidative metabolism, such as lower (*vs.* the first exercise bout [CWR1]) RPE, HR, $\dot{Q}$, R, $\dot{V}$E, the disappearance of the slow component of $\dot{V}O_2$ kinetics and a reduced O_2 cost of exercise, a slightly increased skeletal muscle fractional O_2 extraction and the disappearance of signs of transient unbalance between O_2 delivery and O_2 utilization within skeletal muscles (overshoot). No differences between CWR1 and CWR2 were described when the recovery period was extended to 18 minutes (LONG).

Can the differences mentioned above be considered an expression of a "second wind phenomenon" (1,2,20,46,49), or could they be simply related to a "warm up" or "priming" effect of the first exercise bout, as described also in healthy subjects (see e.g. 4), substantially in terms of a reduced amplitude of the slow component? The answer to this question is not straightforward, but several pieces of evidence appear in favor of a second wind phenomenon. The profound changes described

in the present study in McA during CWR2-SHORT, such as the disappearance of the slow component of $\dot{V}O_2$ kinetics, the substantially lower $\dot{V}E$, HR, $\dot{Q}$, RPE, etc., and the slightly higher fractional O_2 extraction, appear qualitatively and quantitatively quite different from those usually observed in healthy subjects following a priming exercise. Just to make an example, in McA HR values were on average 37 beats $\cdot$ min^{-1} lower during CWR2-SHORT *vs.* CWR1, whereas the priming effect does not usually affect HR values in healthy subjects (see *e.g.* 4). Moreover, in the "control" population represented by MM, which has a similar exercise tolerance compared to McA (see also 14,45) but do not manifest any second wind phenomenon, no differences were observed in CWR2-SHORT *vs.* CWR1. In any case, independently from the definition which is given to the phenomenon, our data demonstrate that in McA a first bout of exercise affects several cardiovascular, ventilatory and metabolic variables, enhances skeletal muscle oxidative metabolism and substantially improves exercise tolerance during a subsequent bout carried out a few minutes after the first. The finding has an obvious clinical interest.

The second wind is usually attributed to an improved delivery of extramuscular energy sources, particularly glucose, to working muscles, following an enhanced sympathoadrenal response (20). The phenomenon has been previously demonstrated in McA patients during prolonged exercise (20,49) or after sucrose administration (1,2,20), and is considered pathognomonic for the disease (49). The second wind has been described in literature as a lower HR (20), lower RPE (48), increased $[C_{(a-v)}O_2]$ (20) and increased [La]b (20) during submaximal exercise, or increased peak work rate and peak $\dot{V}O_2$ (20).

Our results demonstrate that the second wind is also characterized by changes of other physiological variables clearly related to exercise tolerance, such as the slow component of $\dot{V}O_2$ kinetics and the related O_2 cost of exercise (23). As shown in Figure 2 (see CWR1 in McA), the slow component determined a progressive increase in $\dot{V}O_2$ during constant work rate exercise, suggesting a progressive increase in the O_2 cost of exercise, which is directly related with fatigue (23). Our group has recently demonstrated (41) that in obese patients the slope of the slow component is inversely related to the time of exhaustion. In the present study the slow component was eliminated in CWR2-SHORT.

In our study the enhanced exercise tolerance observed in McA during CWR2-SHORT *vs.* CWR1 was associated with a slightly but significantly increased skeletal muscle fractional O_2 extraction (as determined by NIRS), confirming the data obtained by different methods by Haller & Vissing (20). The data demonstrate that the second wind partially corrects the impairment of oxidative

metabolism which is one of the pathophysiological hallmarks of the disease (14,17,20,45,46). Skeletal muscle fractional O_2 extraction in McA, however, remained quite lower than that usually described in healthy subjects (36), as well as in other populations in which skeletal muscle oxidative metabolism is known to be impaired, such as ageing subjects (13), subjects exposed to bed-rest deconditioning (36) or in patients such as heart transplant recipients (27).
In McA the "overshoot" of the Δ[deoxy(Hb+Mb)] kinetics, which was evident during CWR1, disappeared in CWR2-SHORT (but not in CWR2-LONG). According to Ferreira et al. (11), the overshoot is a sign of a relatively inadequate muscle O_2 delivery *vs.* muscle $\dot{V}O_2$, and could lead to a reduced microvascular O_2 pressures and to a lower blood-to-myocyte "driving force" for peripheral O_2 diffusion. The overshoot phenomenon, which suggests an impaired intramuscular matching between O_2 delivery and O_2 utilization, was observed in the present study also in MM, and in previous studies in subjects undergoing bed rest deconditioning (36) and in patients with chronic heart failure (43). In the present study the overshoot disappeared during CWR2-SHORT in McA, but not in MM; this suggests that an improved intramuscular matching between O_2 delivery and O_2 utilization is likely associated with the second wind phenomenon. The possible mechanisms underlying the impaired intramuscular matching between the mentioned variables are discussed in detail in Poole et al. (35), and seem to be related to nitric oxide bioavailability. Also this component of the second wind phenomenon was no longer present after 18 minutes of recovery (CWR2-LONG).

In the present study the work rate of CWR1 and CWR2 cannot be clearly characterized as "moderate" or "heavy" or "severe" (50). As was the case with previous authors (9), in our McA patients we could not determine the gas exchange threshold (GET). It should be remembered that these patients are characterized by the absence of any blood lactate increase during exercise, even at exhaustion, as a consequence of the "blocked" glycogenolysis. GET is usually utilized to discriminate between "moderate" exercise (below GET, with no slow component of $\dot{V}O_2$ kinetics) and "heavy" exercise (above GET, with a slow component which eventually reaches a steady state). In normal subjects, exercises in which the slow component does not reach a steady-state and $\dot{V}O_2$ keeps increasing as a function of time during the constant work-rate exercise (as in McA during CWR1, see Fig. 2), until $\dot{V}O_2$ peak is reached and fatigue ensues, are considered to be in the "severe" exercise domain, above the "critical power" (23). Thus, for McA of the present study the exercise could be defined as "severe" in CWR1 and "moderate" in CWR2-SHORT (24,52).

In conclusion, in the present study carried out on McA patients we demonstrated, for the first time, a "second wind" phenomenon during the second of two consecutive submaximal 6-min constant work rate exercises, separated by 6 minutes of recovery. The second exercise was indeed characterized by significantly lower (compared to the first exercise bout) rate of perceived exertion, heart rate, cardiac output, pulmonary ventilation, gas exchange ratio, and by slightly higher skeletal muscle fractional O_2 extraction. For the first time we also demonstrated that the second wind was associated with signs of enhanced skeletal muscle oxidative metabolism such as the disappearance of slow component of pulmonary $\dot{V}O_2$ kinetics (and therefore with a lower $\dot{V}O_2$ and a lower O_2 cost of exercise), and the disappearance of signs of transient mismatch between O_2 delivery and O_2 utilization in skeletal muscle. We did not observe the second wind phenomenon when the recovery period between the two exercise bouts was longer (18 minutes).

Considering that many activities of everyday life are characterized by bouts of exercise separated by recovery periods, the present results appear of interest also from a clinical and practical point of view. They also give a scientific background to strategies which are often already empowered by McA patients in order to increase their exercise tolerance: for example, having an exercise bout preceded by a few minutes by a "warm up" activity. By following a classic translational approach, the present study applied on patients methods which have been developed in the exercise physiology laboratory, with the ultimate aim of increasing their exercise tolerance and quality of life.

CONFLICT OF INTEREST
The authors declare they do not have conflict of interests.

ACKNOWLEDGEMENTS
The authors thank Mr. Marco Pellegrini and Mrs. Giusi Ferrari for technical assistance.

GRANTS
This study was financially supported by Telethon-UILDM, Project GUP08007

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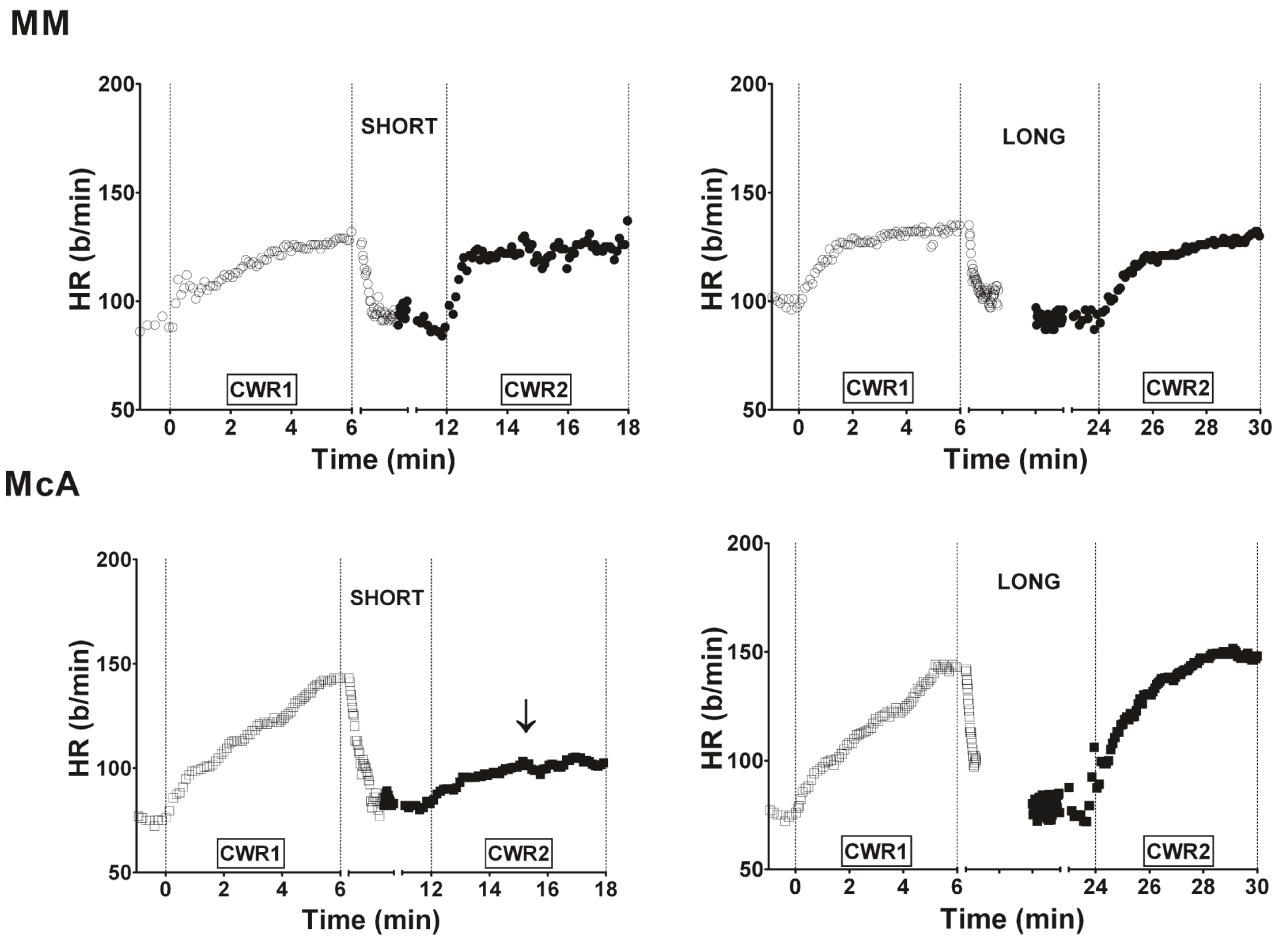

FIGURE 1. Typical individual examples of heart rate (HR) kinetics during the first (CWR1) and the second (CWR2) constant work rate exercise in MM (upper panels) and McA (lower panels). Data obtained with 6 (SHORT) or 18 minutes (LONG) of recovery are presented in the left and right panels, respectively. The vertical hatched lines indicate the transitions from rest to the imposed work rate, and from the imposed work rate to recovery. The second wind phenomenon is indicated by the arrow. See text for further details.

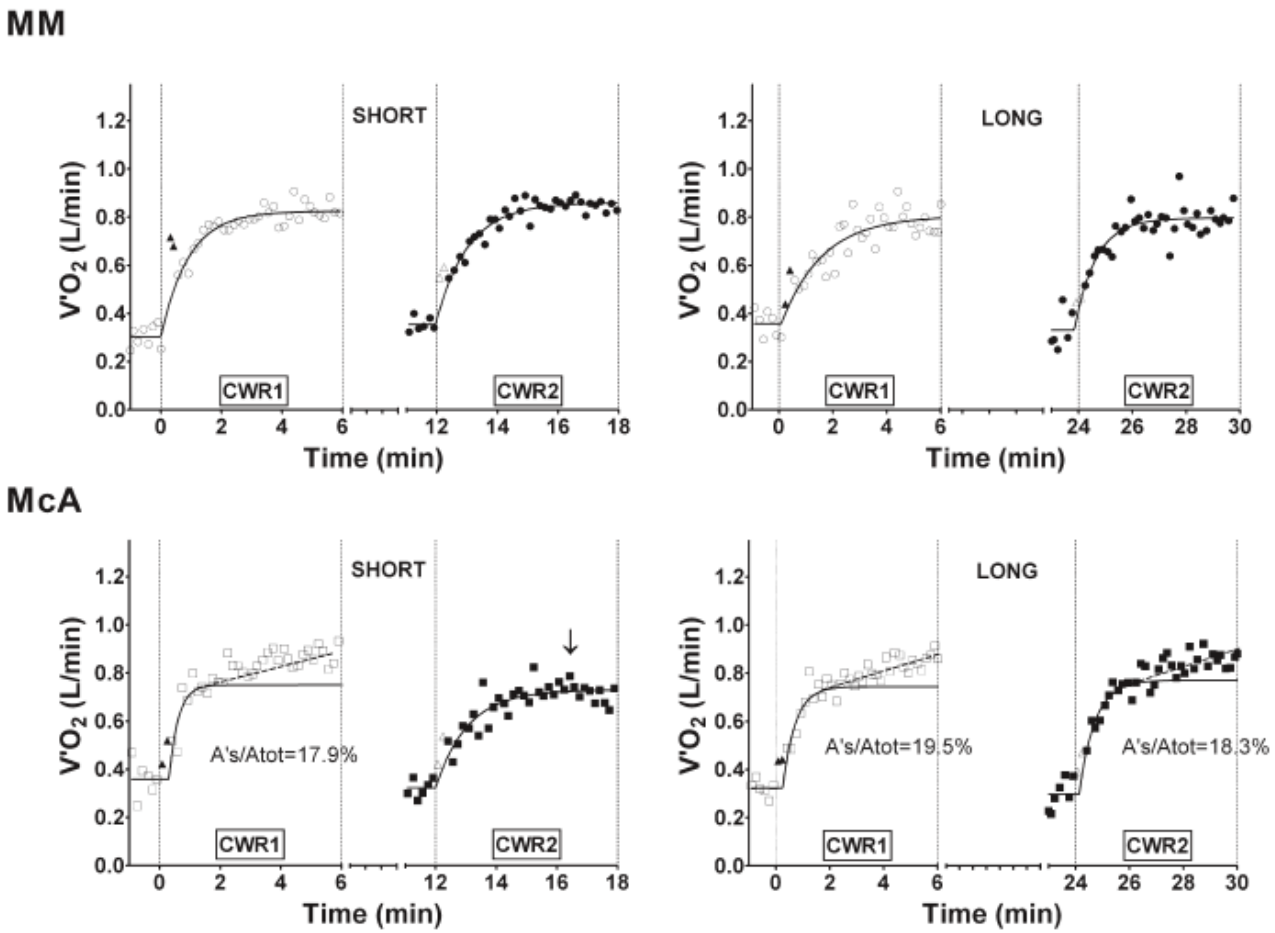

FIGURE 2. Typical individual examples of pulmonary O_2 uptake ($\dot{V}O_2$) kinetics during the first (CWR1) and the second (CWR2) constant work rate exercise in MM (upper panels) and McA (lower panels). Data obtained with 6 (SHORT) or 18 minutes (LONG) of recovery are presented in the left and right panels, respectively. The vertical hatched lines indicate the transitions from rest to the imposed work rate, and from the imposed work rate to recovery. The functions fitting the fundamental component (continuous line) and the slow component (hatched lines) are shown. A'*s*/Atot data are also presented. The second wind phenomenon is indicated by the arrow. See text for further details.

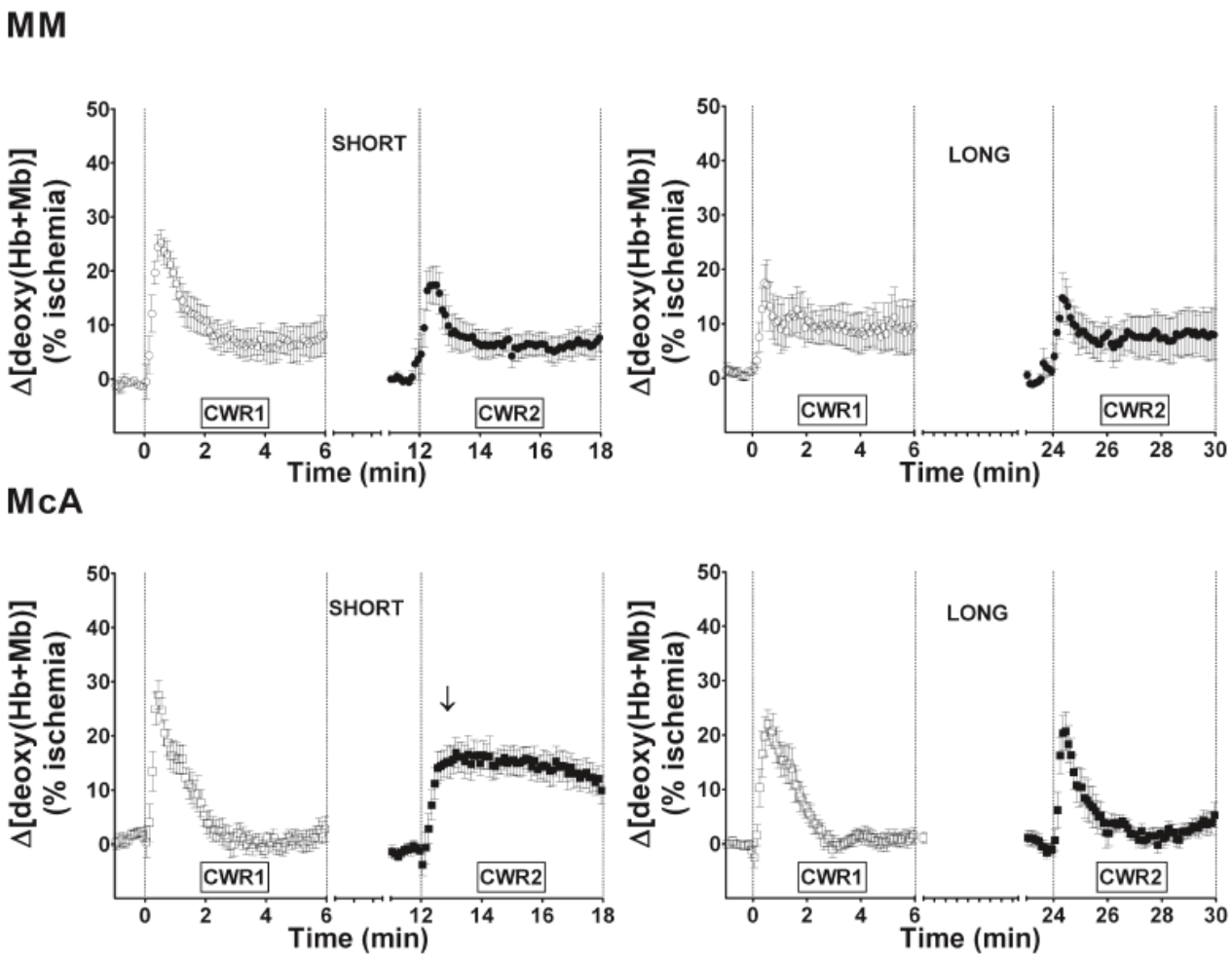

FIGURE 3. Mean (± SD) values every second of skeletal muscle fractional O_2 extraction (Δ[deoxy(Hb+Mb)]) during the first (CWR1) and the second (CWR2) constant work rate exercise in MM (upper panels) and McA (lower panels). Data obtained with 6 (SHORT) or 18 minutes (LONG) of recovery are presented in the left and right panels, respectively. Δ[deoxy(Hb+Mb)] data are expressed as a percentage of Δ[deoxy(Hb+Mb)] changes during ischemia. The vertical hatched lines indicate the transitions from rest to the imposed work rate, and from the imposed work rate to recovery. The second wind phenomenon is indicated by the arrow. See text for further details.

Table 1. *Peak values of the main cardiovascular, ventilatory, and metabolic variables in McA and MM, as well as rates of perceived exertion and blood lactate levels*

	McA	MM
Work rate, W	78.6 ± 18.5	71.7 ± 11.2
RPE	16.6 ± 0.8	15.6 ± 0.7
$\dot{V}O_2$, l/min	1.33 ± 0.25	1.08 ± 0.2
$\dot{V}O_2$, ml·kg^{-1}·min^{-1}	18.5 ± 2.9	15.5 ± 1.1
$\dot{V}CO_2$, l/min	1.22 ± 0.21	1.29 ± 0.2
R	0.93 ± 0.1*	1.21 ± 0.1
$\dot{V}E$, l/min	47.0 ± 6.8	52.4 ± 10.1
VT, liter	1.61 ± 0.2	1.60 ± 0.2
fR, breaths/min	29.4 ± 2.8	31.8 ± 2.3
PET_{O2}, Torr	113.4 ± 2.2	118.7 ± 2.5
PET_{CO2}, Torr	29.8 ± 1.0	30.7 ± 1.7
$[La]_b$, mM	1.2 ± 0.1*	5.8 ± 0.8
HR, beats/min	161.6 ± 3.4*	149.9 ± 8.1
SV, ml	103.2 ± 9.7	109.4 ± 7.5
$\dot{Q}$, l/min	17.5 ± 1.7	16.3 ± 1.2
[a-vCo_2], ml O_2/100 ml	7.6 ± 0.9	6.4 ± 0.7
Δ[deoxy(Hb +Mb)], %ischemia	20.3 ± 8.4	20.1 ± 4.6

Mean (± SD) Values. $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, CO_2 output; R, gas exchange ratio; $\dot{V}E$, pulmonary ventilation; Vt, tidal volume; fR, breathing frequency; $PetO_2$, end-tidal O_2 partial pressure; $PetCO_2$, end-tidal CO_2 partial pressure; [La]b, blood lactate concentration; RPE, rate of perceived exertion; HR, heart rate; SV, stroke volume; $\dot{Q}$, cardiac output; $[C_{(a\text{-}v)}O_2]$, systemic arterial-venous O_2 concentration difference; Δ[deoxy(Hb+Mb)], muscle oxygenation index obtained by NIRS. #$P < 0.05$, significantly different from the corresponding value obtained in MM. See text for further details.

Table 2. *Values of the main cardiovascular, ventilatory, and metabolic variables in CWR1 and CWR2 in McA and MM*

	McA			MM		
	CWR1	CWR2 SHORT	CWR2 LONG	CWR1	CWR2 SHORT	CWR2 LONG
Work rate, W	41.0 ± 14.0	41.0 ± 14.0	41.0 ± 14.0	38 ± 14	38 ± 14	38 ± 14
RPE	13.9 ± 2.6	10.8 ± 1.7*	12.5 ± 1.5	11.7 ± 1.5	12.2 ± 2.0	13.5 ± 3.0
$\dot{V}O_2$, l/min	0.95 ± 0.11	0.86 ± 0.15*	0.94 ± 0.12	0.83 ± 0.09	0.86 ± 0.15	0.84 ± 0.13
$\dot{V}CO_2$, l/min	0.92 ± 0.14	0.81 ± 0.14*	0.89 ± 0.10	0.82 ± 0.16	0.85 ± 0.13	0.83 ± 0.10
R	0.93 ± 0.02	0.86 ± 0.02*	0.90 ± 0.11	0.98 ± 0.11	0.99 ± 0.11	0.98 ± 0.12
$\dot{V}E$, l/min	36.2 ± 3.0	27.6 ± 2.2*	33.8 ± 2.1	30.4 ± 12.1	33.1 ± 11.1	32.2 ± 13.4
$[La]_b$, mM	0.8 ± 0.2	1.2 ± 0.4*	0.9 ± 0.3	3.33 ± 0.41	4.09 ± 0.69	3.65 ± 0.73
HR, beats/min	150 ± 13	113 ± 10*	143 ± 8	115 ± 21.1	122 ± 19.4	124 ± 19.3
SV, ml	102.6 ± 6.5	104.6 ± 5.1	104.7 ± 3.9	97.7 ± 4.4	98.1 ± 5.2	97.9 ± 6.2
$\dot{Q}$, l/min	15.0 ± 0.8	11.6 ± 0.6*	14.8 ± 0.9	11.7 ± 2.0	12.2 ± 2.2	12.5 ± 3.2
[a-vCo_2], ml O_2/100 ml	6.7 ± 0.6	7.7 ± 0.5*	6.5 ± 0.6	6.98 ± 0.87	7.00 ± 0.70	7.02 ± 0.62
Δ[deoxy(Hb+Mb)], %ischemia	−0.1 ± 4.6	14.7 ± 2.3*	1.9 ± 1.0	6.2 ± 3.0	6.0 ± 5.5	6.9 ± 3.5

Mean (± SD) values of $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, CO_2 output; R, gas exchange ratio; $\dot{V}E$, pulmonary ventilation; Gain, $\Delta\dot{V}O_2$ ($\dot{V}O_2$ at the end of CLE minus resting $\dot{V}O_2$) divided by work rate; [La]b, blood lactate concentration; RPE, rate of perceived exertion; HR, heart rate; SV, stroke volume; $\dot{Q}$, cardiac output; $[C_{(a\text{-}v)}O_2]$, systemic arterial-venous O_2 concentration difference; Δ[deoxy(Hb+Mb)], muscle oxygenation index obtained by NIRS. *$P < 0.05$, significantly different from the corresponding value obtained in CWR1. See text for further details.

Table 3. *$\dot{V}o_2$ kinetics parameters for CWR1 and CWR2 in McA and MM*

	τf, s	TDf, s	yBAS, l/min	Af, l/min	$A's$, l/min	$A's/A_{tot}$, %	Gain, ml·min^{-1}·W^{-1}
McA							
CWR1	24.1 ± 4.1	−2.9 ± 3.1	0.30 ± 0.03	0.56 ± 0.07	0.11 ± 0.02	16.0 ± 3.5	16.0 ± 0.5
CWR2 SHORT	29.5 ± 4.5	1.6 ± 2.7	0.33 ± 0.04	0.59 ± 0.06	0.0 ± 0.0*	0.0 ± 0.0*	12.9 ± 0.4*
CWR2 LONG	28.7 ± 3.4	−1.2 ± 1.7	0.32 ± 0.03	0.58 ± 0.07	0.09 ± 0.04	15.7 ± 5.2	14.2 ± 1.5
MM							
CWR1	48.2 ± 11.1	1.1 ± 4.9	0.33 ± 0.03	0.49 ± 0.09	NA	NA	13.3 ± 1.7
CWR2 SHORT	42.4 ± 7.1	−4.5 ± 3.5	0.33 ± 0.04	0.50 ± 0.08	NA	NA	14.9 ± 1.8*
CWR2 LONG	44.7 ± 8.3	−2.9 ± 4.5	0.32 ± 0.03	0.51 ± 0.09	NA	NA	14.2 ± 2.0

Mean (± SD) values of baseline (yBAS); time delay (TDf), time constant (τf), amplitude (Af) of the fundamental component; actual amplitude (A's) of the slow component; and total amplitude of the response (Atot). *P < 0.05, significantly different from the corresponding value obtained in CWR1. NA = not applicable. See text for further details.

EFFECTS OF A HOME-BASED AEROBIC EXERCISE TRAINING IN PATIENTS WITH METABOLIC MYOPATHIES: EVALUATION BY NON-INVASIVE TOOLS SPECIFICALLY AIMED TO MUSCLE OXIDATIVE METABOLISM

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RUNNING TITLE

NIRS and VO_2kinetics in Metabolic Myopathies

FUNDING

This work was supported by a grant from

ABSTRACT

Evidence has been provided that aerobic training should be considered as a treatment for Mitochondrial myopathies (MM) and myophosphorylase deficiency (McArdle's disease, McA). Aim of the present study was to utilize non−invasive methods of functional evaluation, specifically aimed at oxidative metabolism at the skeletal muscle level, in order to evaluate the effects of an aerobic exercise training. 6 MM and 7 McA patients underwent 12 weeks of exercise training (4v/weeks) at home at an heart rate (HR) corresponding to about 65-70% of the maximal HR. Oxygen uptake ($V'O_2$) and skeletal muscle (vastus lateralis) fractional O_2 extraction (by NIRS) were assessed during incremental and moderate and high-intensity constant-load exercises before (BEFORE) and at the end (AFTER) of the training period. $V'O_2$peak increased significantly with training both in MM (from 14.7 ± 3.0 [x ± SD] mL/kg/min BEFORE to 17.6 ± 3.3 AFTER) and in McA (from 18.5 ± 4.7 mL/kg/min to 21.6 ± 5.1 mL/kg/min). Peak skeletal muscle (vastus lateralis) fractional O2 extraction increased with training both in MM (from 22.0 ± 6.7 % to 32.6 ± 5.9) and in McA (from 18.5 ± 6.2 % to 37.2 ± 7.2). The time constants of VO_2 kinetics in moderate-intensity constant-load exercise were significantly reduced in MM (45.1 ± 6.6 s BEFORE vs. 35.9 ± 4.2 s AFTER) and in McA who had presented, before training, markedly slow kinetics of adjustment of VO_2. During the high-intensity constant-load exercise, MM patients showed, after training, clear signs of increased exercise tolerance, such as lower HR (from 134.3 ± 10.8 b/min to 120.8 ± 9.2) and lower scores at the Borg's scale of perceived exertion (from 14.5 ± 1.2 to 12.3 ± 1.5). In MM and McA patients a 12 weeks aerobic training program significantly increased exercise tolerance. Our findings confirm that near infrared spectroscopy and VO_2 kinetics can effectively detect the functional improvements obtained by training, yielding insights also on the mechanisms of the improvements at the pathophysiological level.

KEYWORDS
NIRS, VO2 kinetics, Mitochondrial Myopathies, McArdle's Disease, Training

INTRODUCTION

Metabolic myopathies are an heterogeneous group of disorders characterized by derangements of glycogen or lipid metabolism or mitochondrial function due to genetic mutations leading to defects of the main pathways of energy provision in skeletal muscle fibers. In some of these myopathies, such as myophosphorylase deficiency (McArdle's disease, McA) (Lewis&Haller 1986, Haller &Vissing 2000) or mitochondrial myopathies (MM) (Haller&Vissing 2000, Tarnopolski &Raha 2005, Zeviani &Di Donato 2004) the genetic defect significantly impairs oxidative metabolism. In MM the mutation(s) in the mitochondrial genome (sporadic or maternally inherited) or in the nuclear genome (autosomally inherited) causes the impairment of the mitochondrial respiratory chain and, consequently, a reduced capacity to increase O_2 extraction. In McA the absence or low activity of the myophosphorylase enzyme causes an incapacity to break down intramuscular glycogen, making impossible for the muscle to mobilize glycogen deposits during the exercise. The reduced or absent flux of substrates along the glycolytic pathway impairs one of the two main routes of supply of substrates to the tri−carboxylic acids cycle, and disrupts the delicate interplay between carbohydrate and lipid metabolism. This leads to a significant impairment of oxidative metabolism, to a reduced capacity to increase O_2 extraction during exercise and to a lower than normal maximal aerobic power ($V'O_2$ peak) (REF).

In many MM and McA the impairment of oxidative metabolism lead to a phenotype characterized by a reduced exercise tolerance and easy fatigability, often associated with progressive weakness and myalgia, significantly affecting the patients' quality of life. Nevertheless these patients may not present objective findings on neurological examination such as muscle atrophy or force impairment, and electromyography and routinely laboratory evaluation are frequently normal. Thus, the diagnosis of metabolic myopathy may be difficult and often delayed (Sharp & Haller, 2014) until an invasive procedure like needle biopsy, considered the gold standard diagnostic test, is performed.

A possibility to quantify and serially monitor such impairment by non−invasive tools would be of great interest for clinicians, who need an objective, quantitative and longitudinal evaluation of the impairment to be used in the follow−up of patients, as well as in the assessment of therapies or other interventions. Our group have recently applied to MM and McA two methods of functional evaluation which had been previously utilized in our laboratory on healthy subjects (Grassi et al. 2003), elderly subjects (Ferri et al. 2007), following training (Porcelli et al 2014), on subjects with microgravity−induced muscle atrophy (Lanfranconi et al. 2008) as well as on other types of patients (Borrelli et al. 2003, Lanfranconi et al. 2006) to evaluate oxidative metabolism at the skeletal muscle level. The variables of functional evaluation which we evaluated were (Grassi et al. 2007,

Grassi et al. 2014): A). Skeletal muscle oxygenation indices during exercise, obtained by near−infrared spectroscopy (NIRS) and taken as estimates of the capacity of O_2 extraction. B) Kinetics of adjustment of pulmonary O_2 uptake ($V'O_2$ kinetics) during the transition from rest to exercise.

NIRS is a non−invasive method that allows to monitor muscle oxygenation on the principle that the near−infrared (NIR) light absorption characteristics of haemoglobin (Hb) and myoglobin (Mb) depend on their O2 saturation. Theoretical basis, practical applications, advantages and limitations of NIRS have been extensively reviewed (Ferrari et al. 2004). Briefly, absorption changes of NIR light in muscle reflect changes in oxygenation at the level of small blood vessels (small arterioles and venules), capillaries, and intracellular sites of O2 transport and uptake. The obtained oxygenation indices are the result of the balance between O2 delivery and O2 uptake (V'O2) in the portion of tissue under consideration, being therefore conceptually similar to fractional O2 extraction. In a study (Grassi et al. 2007) conducted on MM and McA we determined by NIRS concentration changes of deoxygenated Hb + Mb (Delta[deoxy(Hb+Mb)]), and the values obtained at exhaustion (Delta[deoxy(Hb+Mb)]peak) during an incremental exercise were taken as an index of the maximal capacity of O_2 extraction. Delta[deoxy(Hb+Mb)]peak was significantly lower in MM and McA compared to controls and Delta[deoxy(Hb+Mb)]peak was also significantly correlated with $V'O_2$ peak. Since the reduced maximal capacity of O_2 extraction represents the key patho−physiological mechanism responsible for the reduced $V'O_2$ peak and exercise tolerance, it is conceivable that in MM and McA Delta[deoxy(Hb+Mb)]peak determined by NIRS can identify and quantify the impairment of oxidative metabolism. A limitation of that approach was represented by the need for the patient to perform an incremental exercise to exhaustion, which may not be feasible in the most compromised patients. Thus, some variables of functional evaluation to be determined during submaximal exercise tests were needed. We hypothesized that a possibility could derive from the determination of the kinetics of adjustment of pulmonary $V'O_2$ during transitions from rest to submaximal exercise (Whipp et al. 2002). It is generally accepted that pulmonary V'O2 kinetics allow a specific evaluation of skeletal muscle oxidative metabolism (Grassi, 2007). This concept should apply even more strictly to patients with metabolic myopathies, in whom the impairment of oxidative metabolism is by definition located in skeletal muscles. In a study conducted by our group on MM and McA (Grassi et al. 2014) $V'O_2$ kinetics were indeed significantly slower (as indicated by the higher time constants) in MM and McA compared to the control groups. Time constants of V'O2 kinetics were significantly and negatively correlated with the NIRS−derived oxygenation index which, as discussed above (Grassi et al. 2007) allows to estimate the maximal capacity of O_2

extraction by skeletal muscle. Thus, slower pulmonary V'O_2 kinetics were associated with an impaired maximal capacity of O_2 extraction.

At present the therapeutic interventions available for patients with metabolic myopathies are very limited (REF.). A possibility could derive from exercise training. As mentioned above, in many MM and McA the reduced exercise tolerance and the associated easy fatigability are often the main symptoms and may significantly affect the patients' quality of life. Recent studies have demonstrated that both in MM (Taivassalo et al. 2001, Taivassalo &Haller 2005, Jeppesen et al. 2006, Taivassalo et al. 2006) and in McA patients (Haller et al. 2006, Mate−Muñoz et al. 2007, Ollivier et al 2005a) moderate−intensity aerobic exercise training is safe and effective in increasing exercise tolerance and in improving quality of life. Taivassalo et al. (2006) demonstrated that 14 weeks of moderate exercise training determined, in MM with single, large−scale mtDNA mutations, no changes in the level of mutated mtDNA, but significantly improved exercise tolerance and peak work capacity. These improvements were associated with an improved capacity for skeletal muscle O_2 extraction. Thus, training affected positively the key patho−physiological mechanism responsible for the disease. Interestingly, in MM the main effect of aerobic training (increased capacity of O_2 extraction) was quite different from that (increased capacity of O_2 delivery by the cardiovascular system) usually described in normal subjects. As for McA, aerobic training significantly increased work capacity, V'O_2peak, peak cardiac output and level of oxidative enzymes in skeletal muscle (Haller et al. 2006). Thus, in McA exercise training would improve exercise tolerance by increasing both the capacity to deliver blood−borne fuels (on which muscle oxidative metabolism critically depends, since glycogen breakdown is blocked) and the capacity by the metabolic machinery to utilize these fuel by oxidative metabolism.

The positive effects of exercise training in patients with metabolic myopathies are further confirmed by the observation that when in MM patients training is interrupted ("deconditioning") the benefits obtained by the previous training regimen substantially disappear (Jeppesen et al. 2006). In addition, two studies have shown that in McA an increase in physical activity level was associated with an amelioration of clinical symptoms and the VO_2peak was 23% higher in physical active patients compared to the inactive patients, supporting the conclusion that physical activity improves exercise capacity (Ollivier et al 2005b, Lucia et al 2012).

The main aim of the present study was to utilize the above−mentioned non-invasive tools of functional evaluation of skeletal muscle oxidative metabolism in order to evaluate the effects of a program of home−based moderate−intensity aerobic exercise training on MM and McA patients. We hypothesized improved exercise tolerance (as evidenced by increases in V'O_2peak and gas exchange threshold) and improved quality of life after training. More specifically, these

improvements should be associated with improvements in the capacity of O_2 extraction by skeletal muscle, determined through the analysis of muscle oxygenation indices obtained by NIRS, and a faster pulmonary $V'O_2$ kinetics during transitions from rest to moderate−intensity exercise. The results will confirm the utility of the proposed non−invasive methods in the follow−up of patients as well as in the evaluation of the effects of therapies or rehabilitation interventions.

MATERIALS AND METHODS

Subjects. 7 patients of both sexes with with myophosphorylase deficiency (McArdle's disease, McA) and 6 patients of both sexes with mitochondrial myopathies (MM) were recruited for the study. Gender distribution, age, and body mass for the two groups of patients were as follows: MM, 4 males (M) and 2 females (F), age (X±SD) = 51±16 yr, body mass = 69.1±18.1 kg; McA, 3 M and 4 F, age = 41±13 yr, body mass = 71.2±22.9 kg. Patients were from the Department of Neuromuscular Diseases, Neurological Institute ''Carlo Besta'' (IRCCS), Milano. The patients were recruited by a Medical Doctor in charge of their clinical assistance. Exclusion criteria were the presence of neoplastic and other major neurological/psychiatric, orthopedic, rheumathologic, endocrine, pulmonary, or cardiovascular disorders. Patients of age <18 and >60 years and patients not capable of performing exercises on a cycloergometer were excluded. The diagnosis of metabolic myopathy was based on clinical, morphological, biochemical, and molecular evaluations. The initial clinical evaluation was carried out in the occasion of the recruitment of the patients. The protocol included a detailed anamnestic evaluation, general physical and neurologic examinations and muscle strength evaluations. Particular enphasis was put on the history related to exercise tolerance, habitual physical activity, signs and symptoms related to exercise and physical activity. Routine hematological examination (including CPK, LDH, transaminase, aldolase), an ECG and a cardiological evaluation was performed. Clinical details for the MM and McA patients were similar to those reported in our previous articles (Grassi et al. 2006 e 2009). Their degree of functional impairment varied from mild—virtually absent (no limitations in activities of everyday life, no significant exercise intolerance)—to severe (very limited exercise tolerance, impairment in activities of daily living). Needle muscle biopsy from quadriceps or deltoid was obtained under local anesthesia and analyzed by histological and hysto−enzymatic reactions (including Oil−Red−O staining for lipid, PAS staining for glycogen, NADH, SDH, Cox staining for mitochondrial analysis). Activities of enzymes of respiratory chain, glycolytic enzymes, and CPT were determined. Molecular biology end genetic tests were also carried out.

The subjects were fully informed of any risk and discomfort associated with the experiments before giving their written consent to participate to the study, which was approved by the ethics

committees of the involved institutions. All procedures were in accordance with the recommendations found in the Declaration of Helsinki (2000) of the World Medical Association.

Study design. This was an open interventional (TRAINING) study in which each patient acted as the control for herself/himself. Each patient was involved in the study for 9 months. During the first 3 month−period (BASELINE) the patients were invited to keep the same level of habitual physical activity they had before entering the study. During the second 3 month−period (TRAINING) the subjects underwent the training protocol (see below for further details). During the third 3 month−period (FOLLOW UP) the patients were free to adjust at their will their level of physical activity. Habitual physical activity and quality of life (see below) were evaluated before (BEFORE) and after (AFTER) the training period, as well as after the post−training follow−up (POST3). The functional evaluation of oxidative metabolism (see below) was performed before (BEFORE) and after (AFTER) the training period.

Exercise training. The training period lasted 12 weeks. The first 1−2 training sessions were conducted in the hospital, under the supervision of a researcher, who was in charge of giving adequate instructions to the patients about the training procedures. The patients conducted the remaining training sessions at home. Training sessions was 4 per week. Each session lasted about 1 hour, and began with about 10−15 minutes of stretching exercises and exercises aimed at optimizing flexibility and balance. These activities were followed by 30 minutes (for the first 6 weeks) or by 45 minutes (for the remaining 6 weeks) of moderate−intensity aerobic training, conducted on a stationary cycloergometer. Patients were allowed to split at their will the 30− or 45−min total daily duration of the training session into combinations of 15−min or 30−min training periods, carried out during the same day. The possibility to split the training session into parts was aimed at enhancing the compliance to the training regimen. Exercise intensity was chosen as to correspond to about 70% of maximal HR, determined for each subject during the incremental exercise described above. HR was displayed and recorded during each exercise session utilizing a Polar NV chest band and watch (Polar Electro, Finland). The cycloergometer recorded on a card the power output vs. time profile of each training session (**Figure 1**). Every week the patients (or someone closed to them) sent these data by e−mail in order to check the compliance and adherence of the patients to the training regimen. The patients were instructed to maintain, during the training period, the same level of physical activity (apart from the training sessions) which characterized the 3 months preceding training (BASELINE). The patients were also instructed to keep a diary for each day of training. All forms of physical activity (type, duration, intensity) outside the training protocol were recorded on the diary. The diary was kept also during the 3 months of pre−training baseline, as well as during the 3 months of follow−up after the completion of the training protocol.

Physical activity Assessment. The physical activity of patients was assessed using the Sensewear Armband (SWA) (BodyMedia Inc., Pittsburgh, USA), a tri-axial accelerometer-based activity monitor coupled with several heat-related sensors (heat flux, body temperature and galvanic skin response). The SWA has been shown to provide valid estimates of energy expenditure during exercise (Johannsen DL, Calabro MA, Stewart J, Franke W, Rood JC, Welk GJ. Accuracy of armband monitors for measuring daily energy expenditure in healthy adults. Med Sci Sports Exerc. 2010; 42 (11): 2134–40) and free-living physical activities (Berntsen S, Hageberg R, Aandstad A, et al. Validity of physical activity monitors in adults participating in free-living activities. Br J Sports Med. 2010; 44 (9): 657–64) in previous studies. The SWA was placed on the patient's right arm over the triceps muscle and worn for three consecutive days. The patients were instructed to continue their normal life while wearing SWA for 24h a day, except while doing water-related activities (i.e showering). Data from SWA were processed using a proprietary software package (v 8.0) and were expressed in $kcal \cdot min^{-1}$.

Quality of life assessment. The quality of life and physical functioning was determined by a validated survey, the Short Form Health Survey Questionnaire (SF−36) (Ware &Sherbourne 1992). SF-36 assesses eight health domains using 35 questions: the questions for the physical function are 10, physical role limitation 4, body pain 2, general health 6, vital force 4, social function 2, psychological role limitation 3 and mental health 5 (Ware &Sherbourne 1992). The range of score was of 0-100. For each of the eight health domains, a scale score was calculated with the higher scores representing better health status. A complete description of the scoring and measurement model is described elsewhere (Garratt et al 1993).

Functional evaluation of oxidative metabolism. The tests were conducted in a laboratory set up in a room of the Istituto Besta. Measurements were carried out during 2 days: on day 1 the patients performed the incremental exercise (see below); on day 2 the patients performed a series of 6−min constant load exercises of moderate intensity. During each testing day the experimental session lasted about 1-1.5 hours. A few minutes before incremental, McA patients ingested 330 mL of caffeine−free drink containing 37 g of sucrose (Andersen et al. 2008), in order to increases exercise tolerance and peak exercise capacity, and abolishes the “second wind” phenomenon occurring after about 10 minutes of exercise (Vissing &Haller 2003).

Exercise protocol. An electromagnetically braked cycloergometer was utilized. Pedalling frequency was digitally displayed to the subjects. Subjects were allowed time to gain familiarity with the investigators and with experimental arrangement, and were carefully instructed about the experimental procedures. Subjects were also familiarized with the experimental protocol by means of short preliminary practice runs. On Day 1, an incremental exercise was performed: after a few

minutes of unloaded pedalling, exercise was conducted at 25−50 W for 6 minutes, and thereafter the workload was increased by 10−25 W (according to the subject's estimated level of physical fitness) every minute until voluntary exhaustion will be reached. The latter was be defined by: 1) inability to maintain the pedalling frequency (60−80 revolutions/min) despite encouragement by the operators; 2) maximal levels of self−perceived exertion, using the validated Borg's scale (Borg 1982); and 3) heart rate (HR) values higher than 85% of the age−predicted maximum. On Day 2, the patients performed 3 repetitions of 6−min constant−load moderate−intensity exercise (CWR-LOW). The work rate was chosen to correspond to about 50% of peak workload reached during the incremental exercise before training. Repetitions were separated by at least 30 min recovery periods. Relatively long recovery periods were chosen a) to be sure that patients were in resting conditions before the next exercise bout; b) to avoid the occurrence of a ''second-wind phenomenon'' in McArdle patients (REF); and c) to avoid a ''priming effect'' on V˙O2 kinetics (REF). On Day 3, MM performed also 1 repetitions of constant−load heavy−intensity exercise at exhaustion(CWR-HIGH). The work rate was chosen to correspond to about 75% of peak workload. Transitions from unloaded pedaling to the imposed load were attained in approximately 3 s.

Measurements. All tests were carried out under close medical supervision, and patients were monitored by 12-lead electrocardiography (ECG). An electromagnetically braked cycle ergometer (Corival, Lode, The Netherlands) was utilized. Pedalling frequency was digitally displayed to the patients. Patients were allowed time to gain familiarity with the investigators and experimental arrangement, and were carefully instructed. Pulmonary ventilation ($\dot{V}E$), $\dot{V}O_2$, and CO_2 output ($\dot{V}CO_2$) were determined breath-by-breath by a computerized metabolic cart (Vmax229; SensorMedics, The Netherlands). Heart rate was determined from the ECG signal. Arterial blood O_2 saturation (SaO_2) was monitored continuously by pulse oximetry (Biox 3740 Pulse Oximeter; Ohmeda, Italy) at the earlobe. Stroke volume was estimated beat-by-beat by impedance cardiography (Physio Flow; Manatec, France). The accuracy of this device has been previously evaluated during incremental exercise in healthy subjects against the direct Fick method and this method has been utilized by our group in other studies (REF.). CO was calculated as HR*SV. At rest and at various time-points (1, 3, 5 and 7 minutes) during recovery after exercise, 20 µl of capillary blood was obtained from a pre-heated earlobe for the determination of blood lactate concentration ([La]b) by an enzymatic method (Biosen 5030; EKF Eppendorf , Italy). The highest [La]b value obtained during recovery was considered [La]b peak.

Oxygenation changes in the vastus lateralis muscle were evaluated by near-infrared spectroscopy (NIRS) (Boushel R, Langberg H, Olesen J, Gonzales-Alonso J, Bülow J, Kjær M. Monitoring tissue oxygen availability with near infrared spectroscopy in health and disease. Scand J Med Sci Sports

11: 213–222, 2001, Ferrari M, Mottola L, Quaresima V. Principles, techniques, and limitations of near-infrared spectroscopy. Can J Appl Physiol 29: 463–487, 2004). A portable NIR continuous-wave photometer (PortaMon, Artinis, The Netherlands) was utilized. Specific details on the method can be found in recent papers by our group (Porcelli S, Marzorati M, Lanfranconi F, Vago P, Pišot R, Grassi B. Role of skeletal muscles impairment and brain oxygenation in limiting oxidative metabolism during exercise after bed rest. J Appl Physiol 109:101–111, 2010, Porcelli S, Marzorati M, Belletti M, Bellistri G, Morandi L, Grassi B. The "second wind" in McArdle's disease patients during a second bout of constant work rate submaximal exercise. J Appl Physiol 116: 1230–1237, 2014). The instrument measures micromolar (μM) changes in oxygenated hemoglobin (Hb) + myoglobin (Mb) concentrations (Δ[oxy(Hb+Mb)]), and in deoxygenated [Hb + Mb] (Δ[deoxy(Hb+Mb)]), with respect to an initial value arbitrarily set equal to zero and obtained during the resting condition preceding the test. Δ[deoxy(Hb+Mb)] is relatively insensitive to changes in blood volume and has been considered an estimate of skeletal muscle fractional O2 extraction (ratio between O2 consumption and O2 delivery) (Ferreira LF, Poole DC, Barstow TJ. Muscle blood flow-O2 uptake interaction and their relation to on-exercise dynamics of O2 exchange. Respir Physiol Neurobiol 147: 91–103, 2005). Reliability of tissue oxygenation indexes obtained by NIRS, evaluated by the intraclass correlation coefficient for repeated measurements on the same subject during different days, was found to be very high for skeletal muscle (Subudhi AW, Dimmen AC, Roach RC. Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise. J Appl Physiol 103: 177–183, 2007). A "physiological calibration" of Δ[deoxy(Hb+Mb)] values was performed by obtaining a transient ischemia of the limb after the exercise period (subject in the sitting position on the cycloergometer): data obtained during exercise were expressed as a percentage of the values of maximal muscle deoxygenation obtained by pressure cuff inflation (at 300–350 mmHg), carried out at the inguinal crease of the thigh for a few minutes until Δ[deoxy(Hb+Mb)] increase reached a plateau (Grassi B, Pogliaghi S, Rampichini S, Quaresima V, Ferrari M, Marconi C, Cerretelli P. Muscle oxygenation and gas exchange kinetics during cycling exercise on-transition in humans. J Appl Physiol 95:149–158, 2003).

Kinetics analysis. $\dot{V}$O2 kinetics were evaluated during transitions from unloaded pedaling to constant load exercise. Breath-by-breath $\dot{V}$O2 values obtained in the various repetitions of the exercise were time aligned and then superimposed for each subject. Average $\dot{V}$O2 values every 10 s were calculated. Data obtained during the first 20 s of the transition (''cardiodynamic'' phase [Whipp BJ, Rossiter HB, Ward SA. Exertional oxygen uptake kinetics: a stamen of stamina? Biochem Soc Trans. 2002;30:237–47]) were excluded from analysis. Thus, $\dot{V}$O2 kinetics analysis

dealt mainly with the ‘‘phase 2’’ (or ‘‘fundamental’’ component) of the response, which more closely reflects gas exchange kinetics occurring in skeletal muscles (Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK, Wagner PD. Muscle O2 uptake kinetics in humans: implications for metabolic control. J Appl Physiol. 1996;80:988–98). To evaluate mathematically the $\dot{V}O2$ kinetics, data were fitted by the function:

$$y(t) = yBAS + A_f [-e^{(t-TDf)/\tau f}], \qquad [1]$$

and parameter values (TDf, τf) were determined that yielded the lowest sum of squared residuals. In equation 1, yBAS indicates the baseline; A_f is the amplitude between the yBAS and the steady state during the fundamental component; and TD_f is the time delay and τ_f is the time constant of the function for the fundamental component. To check the presence of a ‘‘slow component’’ (Whipp BJ, Rossiter HB, Ward SA. Exertional oxygen uptake kinetics: a stamen of stamina? Biochem Soc Trans. 2002;30:237–47) of the kinetics, data were also fitted by the function:

$$y(t) = yBAS + A_f [-e^{(t-TDf)/\tau f}] + A_s [-e^{(t-TDs)/\tau s}] \quad [2]$$

In equation 2, A_s, TD_s, and T_s indicate, the amplitude, the time delay, and the time constant of the slow component, respectively.

Statistical analysis. Results were expressed as mean values ± Standard Deviation (x ± SD). Comparisons between patients before and after the training program were performed by two−sided Student’s t−test. Comparisons between more than two observations were performed by one−way ANOVA; a Tukey’s post−hoc test was utilized when significant differences emerged at ANOVA. Data fitting by linear regression or by other functions was performed by the least squared residuals method. Comparisons between fittings with different functions models were performed by F test. The level of significance was set at $P<0.05$. Statistical analyses were performed by a software package (Prism 5.0, GraphPad).

RESULTS

Training. Overall the compliance to the training regimen was rated as "very good". The work rate of the first two (START) and the last three (END) training sessions that patients carried at their homes is shown in **Figure 2**. There was a significantly increase of the mean work rate of the training sessions in both MM and McA.

Incremental exercise. Peak values of the main respiratory, cardiovascular and metabolic variables are shown in **Table 1**. In McA and MM, values obtained before training were very similar to those obtained in a previous study by our group (14) and by others (19,21). After training, an increase of ~20% in work rate and $\dot{V}O_2$ peak values were observed both in MM and McA, indicating a severely improved exercise capacity. SV and $\dot{Q}$ peak values were also significantly increased both in McA and MM, reaching values slightly lower than those usually obtained in healthy controls (39). HR peak were significantly higher in McA vs. MM, corresponding respectively to ~83% and ~96% of the age–predicted maximum. Aerobic training also improved peak skeletal muscle fractional O_2 extraction capacity, even if values were very low for both patients groups. As expected, in McA R peak values were relatively low, and [La]b peak values were not higher than those determined at rest (1.2 ± 0.1 mM). For the other variables no differences were observed after training both in McA and MM.

Moderate-intensity constant work rate exercise. Mean (± SD) values determined in the last ~30 s of CWR-LOW are presented in **Table 2**. $\dot{V}O_2$, $\dot{V}E$, HR, $\dot{Q}$ and RPE values were significantly reduced after training, both in MM and McA. On the other hand, Δ[deoxy(Hb+Mb)] was significantly higher in AFTER vs. BEFORE. For the other variables no differences were observed between BEFORE and AFTER both in MM and McA.

Heavy-intensity constant work rate exercise. Figure 3 shows $\dot{V}O_2$, $\dot{Q}$ and Δ[deoxy(Hb+Mb)] values BEFORE and AFTER training in MM. After training, $\dot{V}O_2$ was significanly reduced. A significant reduction was also observed for $\dot{V}CO_2$ (1.12±0.2 in BEFORE and 0,98±0.1 in AFTER), $\dot{V}E$ (45.2±7,6 in BEFORE and 38.5±6.8 in AFTER) and R (1.1±0.1 in BEFORE and 1.0±0.1 in AFTER) values. As for cardiovascular response to exercise, HR values at the end of CWR-HIGH were lower (by about 10 beats · min^{-1}) in AFTER (120.8±9.2 b*min^{-1}) than in BEFORE

(134.3±10.8 b*min^{-1}). $\dot{Q}$ values were also significantly reduced (Fig. 3). On the contrary, peak skeletal muscle fractional O_2 extraction capacity significantly increased after training. In AFTER, the time of exhaustion (10,0±0,6 min) was slightly higher than BEFORE (9,2±0,7 min) and a lower scores at the Borg's scale of perceived exertion was observed (from 14.5 ± 1.2 to 12.3 ± 1.5).

$\dot{V}O_2$ kinetics. Typical individual examples of $\dot{V}O_2$ kinetics of a MM (upper panels) and of a McA (lower panels) during CWR-LOW are shown in **Figure 4**. In MM, a slow component was not observed both in BEFORE and AFTER. As for McA, $\dot{V}O_2$ values did not reach a steady-state and a slow component was evident BEFORE. The parameters of $\dot{V}O_2$ kinetics are shown in **Table 3**. In MM τf and Af values were significantly reduced in AFTER *vs.* BEFORE. In McA TDf, τf and Af values were not affected by training. However, a slow component, corresponding to ~16 % of the total amplitude of the response, was present in all McA in BEFORE. After training, the amplitude of the slow component was significantly reduced in two patients and it disappeared in the remaining five patients. Gain values (G) were calculated as $\Delta\dot{V}O_2$ ($\dot{V}O_2$ at the end of CWR minus resting $\dot{V}O_2$) divided by work rate. In both groups of patients, G values were significantly reduce by training intervention even if they remained substantially higher than those usually observed in normal subjects (~10 ml · min^{-1} · watt^{-1}).

Daily physical activity. TEE was not different BEFORE (36.6 ± 9.2 kcal/day/kg) vs. AFTER (35.7 ± 13.4) training. TEE values corresponded to a "low" level of habitual physical activity according to standard classifications.

DISCUSSION

Mitochondrial myopathies and McArdle's disease are genetic disorders characterized by impairments of energy metabolism which translate into reduced exercise tolerance and easy fatigability. In two studies our group have demonstrated that two non−invasive methods of functional evaluation specifically aimed to oxidative metabolism at the skeletal muscle level are able to characterize the metabolic impairment of these patients. In particular, we showed that, skeletal muscle oxygenation indices during exercise, obtained by near−infrared spectroscopy and taken as estimates of the capacity of O_2 extraction, allows to quantify, noninvasively, the limitation to skeletal muscle oxidative metabolism both in MM and McA patients. Moreover, the kinetics of adjustment of pulmonary VO_2 during transitions to constant-load moderate-intensity exercise were

quantitatively correlated with an impaired peak capacity of O2 extraction and with a lower peak aerobic power. At present the therapeutic interventions available for these patients are very limited. Evidence from the literature suggests that moderate−intensity aerobic exercise training represents a safe intervention, which may benefit MM and McA patients by increasing their exercise tolerance. In this study, 6 MM and 7 McA patients underwent 12 weeks of home-based exercise training: 4 training sessions per week; about 1 hour per session; 30-45 minutes of moderate-intensity aerobic exercise on a cycle ergometer, at an heart rate (HR) corresponding to about 65-70% of the maximal HR. After training, peak O2 uptake (V'O2peak), variable evaluating maximal aerobic power, increased significantly both in MM and in McA. The 20% increase in V'O2peak, which is similar to that usually described following similar training programs in healthy subjects, demonstrates an increased exercise tolerance. Peak skeletal muscle (vastus lateralis) fractional O2 extraction, as estimated by near-infrared spectroscopy (NIRS), increased with training both in MM and in McA. From these data it can be inferred that the increased V'O2peak with training was attributable, at least in part, to an increased skeletal muscle fractional O2 extraction. In a previous study (Grassi et al., Muscle Nerve 35: 510-520, 2007) we had demonstrated that the impaired fractional O2 extraction by skeletal muscles, as evaluated by NIRS, represents a key pathophysiological factor both in MM and in McA patients.

Before and at the end of the training period the patients carried out in the lab also constant work rate exercises on a cycle ergometer, at moderate intensity (about 50% of V'O2peak) and at heavy intensity (65-70% of V'O2peak). During the latter MM patients showed, after training, clear signs of increased exercise tolerance, such as lower HR (from 134.3 ± 26.6 b/min to 121.2 ± 21.9), lower gas exchange ratio (from 1.11 ± 0.16 to 1.02 ± 0.10), lower scores at the Borg's scale of perceived exertion (from 14.5 ± 1.2 to 12.3 ± 1.5).

In a previous study we had observed in MM and in McA patients slower than normal pulmonary "V'O2 kinetics" (Grassi et al., Med. Sci. Sports Exerc. 41: 2120-2127, 2009) (the term indicates the rate of adjustment of pulmonary V'O2 during rest to constant work rate transitions). As pointed out in another previous paper by our group (Grassi et al., Eur. J. Appl. Physiol. 111: 345-355, 2011), faster V'O2kinetics reflect a better performance of skeletal muscle oxidative metabolism, and are associated with lower "O2 deficit" and higher exercise tolerance. We hypothesized that if training obtained (as we were expecting) an improvement of skeletal muscle oxidative metabolism, we would see faster V'O2 kinetics in the patients after training. We were rather surprised to see only non-significant decreases, after training, of the parameter (time-constant [a lower time-constant indicates a faster kinetics]) which we utilized to evaluate the V'O2 kinetics: the time constants were

indeed 42.3 ± 17.4 s before training vs. 36.9 ± 10.2 s after training in MM (P=0.18), and 39.0 ± 31.3 s before training vs. 31.9 ± 10.1 after training in McA (P=0.54). However, after we took into consideration the 4 patients with a markedly slower V'O2 kinetics before training (as an arbitrary cutoff we considered a time-constant of at least 45 s [values in healthy subjects are in the range of 25-35 s]), we observed after training values (39.7 ± 8.5 s) which were significantly lower than those obtained before training (67.7 ± 22.3). In other words, training significantly speeded the V'O2 kinetics only in the patients who had presented, before training, markedly slow V'O2 kinetics. This concept appears compatible with the results of our previous study (Grassi et al., Med. Sci. Sports Exerc. 41: 2120-2127, 2009), in which we showed markedly slower V'O2 kinetics only in the patients with the most pronounced metabolic impairment. In other words, we demonstrated that the analysis of V'O2 kinetics is a valuable functional evaluation tool of the effects of training in MM and McA patients with severe metabolic impairment.

The habitual level of physical activity during the day was evaluated by estimating the daily total energy expenditure (TEE). Before and about 2 months after the termination of the training period the subjects wore a dedicated device (SenseWear Armband) during 3 typical consecutive days. TEE was not different before (36.6 ± 9.2 kcal/day/kg) vs. after (35.7 ± 13.4) training; TEE values corresponded to a "low" level of habitual physical activity according to standard classifications. In other words, the adopted exercise training program, which increased exercise tolerance of the patients, did not induce, a couple of months after the termination of the training program, an increased level of habitual physical activity. We have no specific data to explain this unexpected and rather disappointing finding.

In conclusions, in MM and McA patients a standardized training program with aerobic exercise of moderate intensity, supervised but carried out at home autonomously by the patient, significantly increases exercise tolerance. We also demonstrated that a combination of traditional and more innovative functional evaluation methods (identified in previous work by our group) can effectively detect the functional improvements obtained by training, yielding insights also on the mechanisms of the improvements at the pathophysiological level. Surprisingly, the improvements in exercise tolerance obtained by the training program did not determine an increase in the habitual level of physical activity evaluated a couple of months after the termination of the training program. Further studies should be addressed to evaluate specific "innovative" interventions that can also stimulate patients at a "motivational" level in order to obtain an increase in the level of habitual physical activity.

CONFLICT OF INTEREST
The authors declare they do not have conflict of interests.

ACKNOWLEDGEMENTS
The authors thank Mr. Marco Pellegrini and Mrs. Giusi Ferrari for technical assistance.

GRANTS
This study was financially supported by Telethon-UILDM, Project GUP08007

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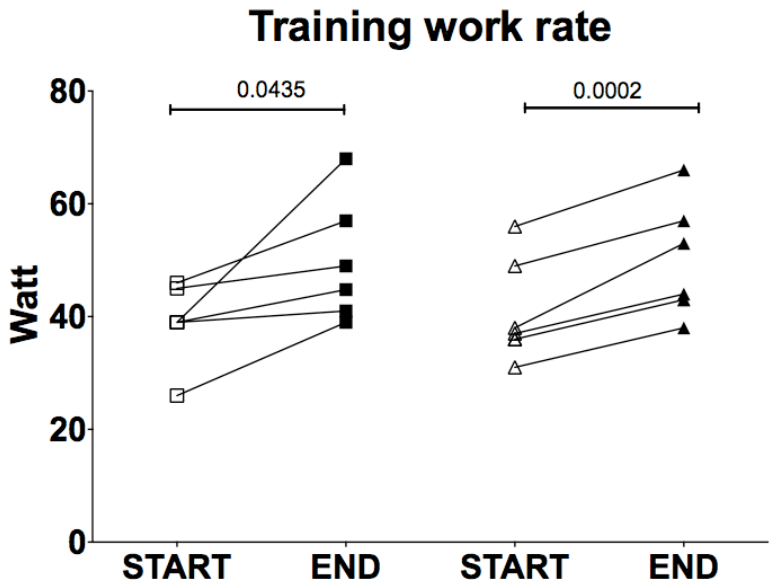

FIGURE 2. Individual work rate (watt) recorded by the bicycle during the first (START) and the last three (END) sessions of training.

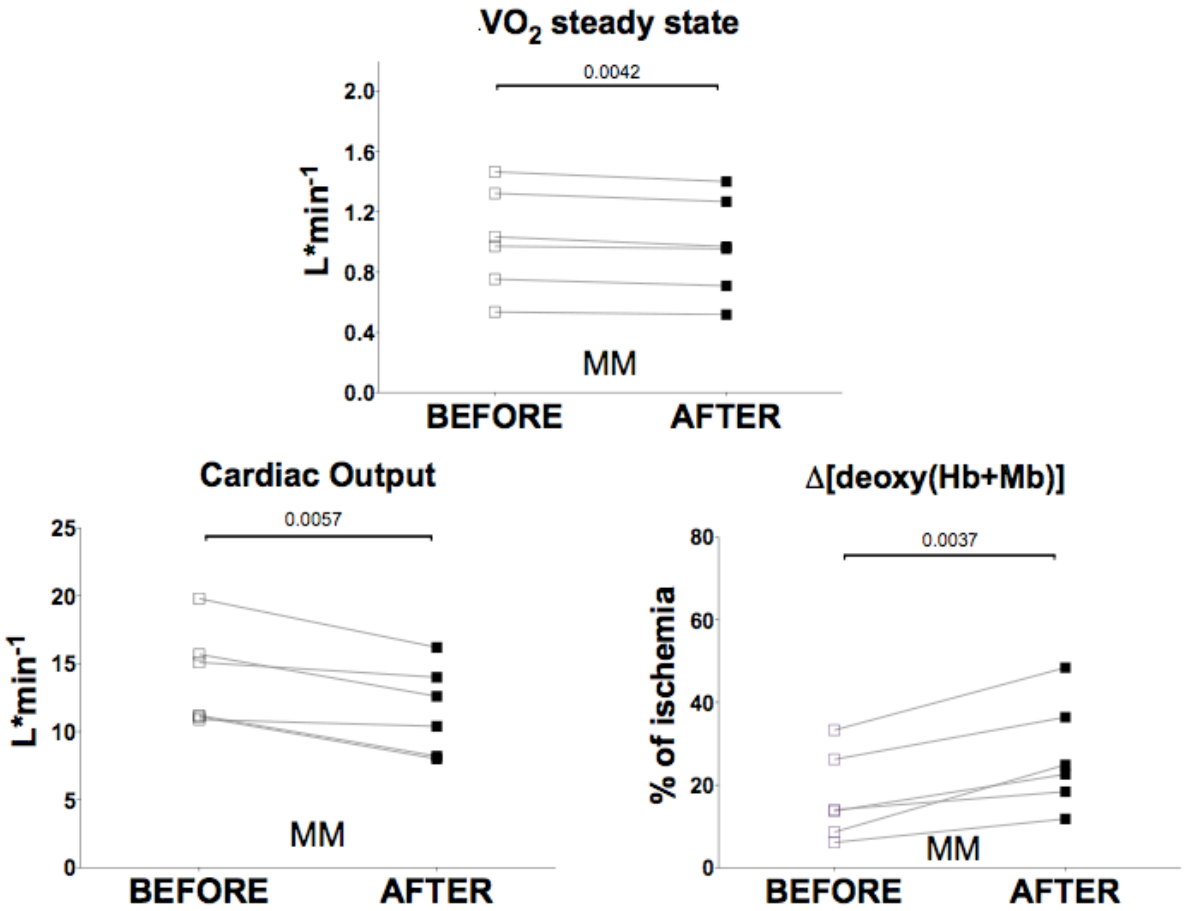

FIGURE 3. Individual values of oxygen consumption (VO_2), cardiac output (Q) and peak skeletal muscle fractional O_2 extraction capacity (Δ[deoxy(Hb+Mb)]) during the heavy-intensity constant work rate exercise in MM. Data obtained before (BEFORE) and after (AFTER) training are presented

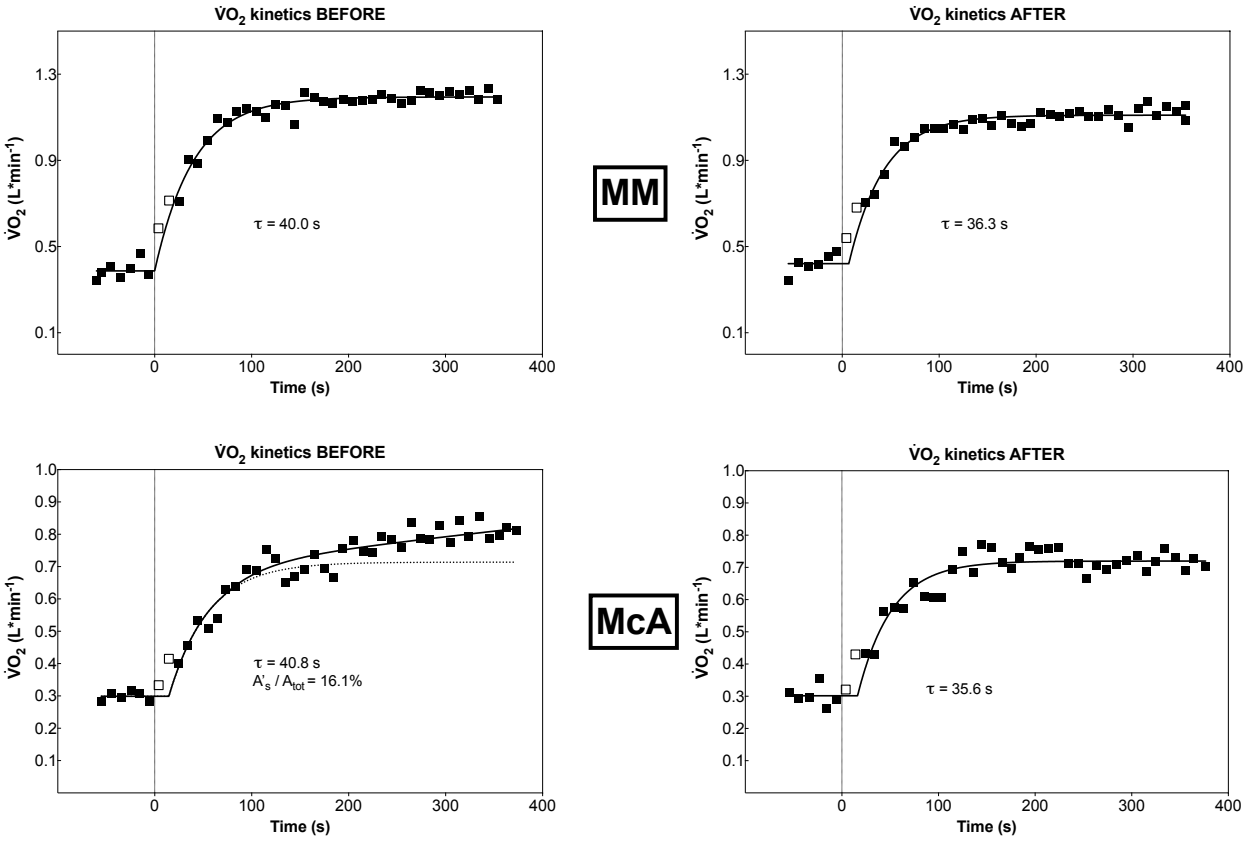

FIGURE 4. Typical individual examples of pulmonary O_2 uptake ($\dot{V}O_2$) kinetics during the moderate-intensity constant work rate exercise in MM (upper panels) and McA (lower panels). Data obtained before (BEFORE) and after (AFTER) training are presented in the left and right panels, respectively. The vertical hatched lines indicate the transitions from rest to the imposed work rate. The functions fitting the fundamental component (hatched line) and the slow component (continuos lines) are shown. A'*s*/Atot data are also presented. See text for further details.

Table 1. Main respiratory, cardiovascular and metabolic variables in MM and McA. Peak values obtained during the incremental exercise both before and after 12 weeks of aerobic training

Patient	Work rate	RPE	$V'O_2$	$V'O_2$	$V'CO_2$	R	V'E	Vt	fR	$PetO_2$	$PetCO_2$	[La]b	HR	SV	Q'	Δ[deoxy(Hb +Mb)]
	Watt		$L \cdot min^{-1}$	$ml \cdot kg^{-1} \cdot min^{-1}$	$L \cdot min^{-1}$		$L \cdot min^{-1}$	L	$b \cdot min^{-1}$	mmHg	mmHg	mM	$b \cdot min^{-1}$	mL	$L \cdot min^{-1}$	% of ischemia
MM BEFORE	71.7 ± 13.0	15.8 ± 0.7	1.06 ± 0.17	14.7 ± 1.2	1.21 ± 0.22	1.17± 0.1	48.6 ± 9.9	1.53 ± 0.2	30.7 ± 2.3	117.5 ± 2.5	31.7 ± 1.8	5.7 ± 0.7	141.2 ± 12.1	106.5 ± 8.6	14.9 ± 1.5	22.0 ± 6.7
MM AFTER	87.5 ± 14.5#	16.7 ± 0.8	1.22 ± 0.19#	17.6 ± 1.4	1.43 ± 0.24#	1.17 ± 0.1	55.9 ± 12.4	1.75 ± 0.2	30.6 ± 3.4	117.0 ± 3.8	33.3 ± 3.0	6.8 ± 1.2	141.0 ± 13.6	108.7 ± 4.6	15.1 ± 1.2	32.6 ± 5.9#
McA BEFORE	72.9 ± 12.6	16.1 ± 0.8	1.29 ± 0.15	18.5 ± 1.8	1.23 ± 0.12	0.98 ± 0.1	49.2 ± 4.0	1.66 ± 0.2	30.4 ± 2.4	115.8 ± 2.6	29.1 ± 1.7	1.1 ± 0.1	170.6 ± 4.6	89.7 ± 8.1	15.2 ± 1.3	18.5 ± 6.2
McA AFTER	89.3 ± 11.8#	14.6 ± 0.7	1.48 ± 0.15#	21.6 ± 1.9#	1.33 ± 0.14#	0.90 ± 0.1	50.5 ± 4.7	1.65 ± 0.2	31.2 ± 2.1	112.2 ± 2.4	31.2 ± 1.6	1.2 ± 0.1	172.4 ± 5.5	109.6 ± 5.6#	18.9 ± 1.1#	37.2 ± 7.2#

Mean (± SD) Values. $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, CO_2 output; R, gas exchange ratio; $\dot{V}E$, pulmonary ventilation; Vt, tidal volume; fR, breathing frequency; $PetO_2$, end-tidal O_2 partial pressure; $PetCO_2$, end-tidal CO_2 partial pressure; [La]b, blood lactate concentration; RPE, rate of perceived exertion; HR, heart rate; SV, stroke volume; $\dot{Q}$, cardiac output; $[C_{(a-v)}O_2]$, systemic arterial-venous O_2 concentration difference; Δ[deoxy(Hb+Mb)], muscle oxygenation index obtained by NIRS. #P < 0.05, significantly different from the corresponding value obtained BEFORE. See text for further details.

Table 2. Main respiratory, cardiovascula and metabolic variables in MM and McA. Mean (±SD) values obtained during the moderate-intensity constant load exercise both before and after 12 weeks of aerobic training.

Patient	Work rate	RPE	$V'O_2$	$V'O_2$	$V'CO_2$	R	V'E	[La]b	HR	SV	Q'	Δ[deoxy(Hb +Mb)]
	%Wmax		$L \cdot min^{-1}$	$ml \cdot kg^{-1} \cdot min^{-1}$	$L \cdot min^{-1}$		$L \cdot min^{-1}$	mM	$b \cdot min^{-1}$	mL	$L \cdot min^{-1}$	% of ischemia
MM BEFORE	54.2 ± 5.4	12.1 ± 0.6	0.86 ± 0.10	12.5 ± 0.6	0.84 ± 0.12	0.99± 0.1	32.3 ± 4.5	3.7 ± 0.3	120.0 ± 7.9	99.2 ± 3.9	11.8 ± 0.8	12.2 ± 5.0
MM AFTER	44.9 ± 5.7#	10.8 ± 0.5#	0.79 ± 0.10#	11.5 ± 0.6#	0.76 ± 0.09	0.95 ± 0.1#	27.1 ± 4.0#	3.1 ± 0.7	102.9 ± 6.4#	93.7 ± 3.7	9.6 ± 0.6#	18.7 ± 4.3#
McA BEFORE	59.7 ± 4.7	13.6 ± 0.6	0.95 ± 0.15	13.7 ± 1.2	0.85 ± 0.13	0.91 ± 0.1	36.2 ± 4.0	0.9 ± 0.1	150.8 ± 3.8	101.4 ± 7.4	15.2 ± 0.8	-3.5 ± 5.7
McA AFTER	46.7 ± 3.8#	11.5 ± 0.8#	0.86 ± 0.16#	12.6 ± 1.3#	0.74 ± 0.13	0.85 ± 0.1	26.9 ± 3.3#	1.0 ± 0.1	131.1 ± 7.0#	100.3 ± 6.9	13.0 ± 0.9#	10.1 ± 5.1#

Mean (± SD) values of $\dot{V}O_2$, oxygen uptake; $\dot{V}CO_2$, CO_2 output; R, gas exchange ratio; $\dot{V}E$, pulmonary ventilation; Gain, $\Delta\dot{V}O_2$ ($\dot{V}O_2$ at the end of CLE minus resting $\dot{V}O_2$) divided by work rate; [La]b, blood lactate concentration; RPE, rate of perceived exertion; HR, heart rate; SV, stroke volume; $\dot{Q}$, cardiac output; Δ[deoxy(Hb+Mb)], muscle oxygenation index obtained by NIRS. #P < 0.05, significantly different from the corresponding value obtained in BEFORE. See text for further details.

Table 3. V'O_2 kinetics parameters for CWR-LOW in MM and McA. Data obtained before (BEFORE) and after 12 weeks of training (AFTER) are shown.

		τf	TD*f*	y*BAS*	A*f*	A'*s*	A's/ Atot	Gain
		s	s	$L \cdot min^{-1}$	$L \cdot min^{-1}$	$L \cdot min^{-1}$	%	$mL \cdot min^{-1} \cdot watt^{-1}$
MM	BEFORE	45.1 ± 6.6	1.9 ± 3.7	0.31 ± 0.03	0.55 ± 0.10	NA	NA	15.1 ± 1.3
	AFTER	35.9 ± 4.2#	2.3 ± 4.9	0.31 ± 0.05	0.48 ± 0.11#	NA	NA	13.0 ± 0.7#
McA	BEFORE	36.7 ± 2.1	2.7 ± 2.5	0.31 ± 0.03	0.55 ± 0.06	0.09 ± 0.03	17.0 ± 4.4	16.2 ± 0.9
	AFTER	33.2 ± 1.9	6.4 ± 2.9	0.29 ± 0.03	0.53 ± 0.05	0.04 ± 0.03#	6.4 ± 4.3#	13.9 ± 0.9#

Mean (± SD) values of baseline (y*BAS*); time delay (TD*f*), time constant (τf), amplitude (A*f*) of the fundamental component; actual amplitude (A'*s*) of the slow component; and total amplitude of the response (Atot); Gain, $\Delta \dot{V}O_2$ ($\dot{V}O_2$ at the end of CWR minus resting $\dot{V}O_2$) divided by work rate. *P < 0.05, significantly different from the corresponding value obtained in BEFORE. NA = not applicable. See text for further details.

FINAL CONSIDERATIONS

The results of the studies reported in this thesis demonstrate that, in patients with metabolic myopathies, a functional evaluation of oxidative metabolism carried out at the whole-body and at the skeletal muscle level allows to quantify the metabolic impairment and to identify the improvements in exercise tolerance following therapies and/or rehabilitation interventions. At the same time, the analysis of the physiological and bioenergetic adaptations to exercise in these patients offers to physiologists the opportunity to better understand the regulation of basic physiological processes and their relationship with systemic adaptations to exercise. In other words, the studies presented in this thesis followed a translational approach, "from the research lab to the bed side", applying some methods of functional evaluation of oxidative metabolism, which our group and others have developed over the years, to patients with metabolic myopathies. The results appear of interest if one considers the expanding role of exercise training as a therapeutic intervention in patients with metabolic myopathies. Thus, an integrated/translational approach which combines traditional clinical examination and more innovative functional evaluation methods should be pursued to gain mechanistic insights into the pathophysiology of these diseases and into the effects of therapeutical/rehabilitative interventions.

The "next steps" will be to evaluate if, in patients with late-onset Pompe disease, a standardized home-based exercise training program of moderate-intensity (similar to that utilized in Mitochondrial myopathies and McArdle disease and described above) can delay or reverse, at least in part, the reduced efficacy of enzyme replacement therapy often described after a few years of administration. This study has just started. Moreover, it could be interesting to study the effects of dietary interventions on exercise tolerance and quality of life of patients with metabolic myopathies. Specifically, we plan to investigate if a hyperproteic-low carbohydrate diet in patients with Pompe disease could reduce glycogen deposition and increase the intracellular protein synthesis, thereby reducing glycogen accumulation, proteolysis, muscle autophagy and damage. Secondary, we plan to determine the efficacy of a short-term (6 days) dietary supplementation of nitrate, a natural constituent of the human diet (spinach, beetroot, etc.) which induces vasodilation and thereby decreases blood pressure by nitric oxide production, in increasing the metabolic efficiency and exercise tolerance in patients with McArdle disease and late-onset Pompe disease. Indeed, recent studies indicate that dietary nitrate decreases the O_2 cost of exercise and improves intramuscular O_2 delivery-O_2 utilization matching, increasing exercise tolerance. These effects would be extremely helpful in patients with McArdle or Pompe disease, in whom the reduced exercise tolerance is associated with a markedly increased (compared to healthy control subjects) O2 cost of exercise.

In conclusion, contemporary biomedical sciences need an integrated and multidisciplinary approach. For the development and implementation of biomedical knowledge, investigators working in clinical medicine must perform reverse translational investigations at the cellular and molecular level. Similarly, physiologists performing basic research must look for opportunities to translate their findings to the whole organism, both healthy and pathological.

OTHER PUBBLICATIONS

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AKNOWLEDGEMENTS

This thesis has been a unique journey and been seen through to completion with the support and encouragement of numerous people. A special thanks goes to my family. Words can not express how grateful I am to my father for all of the sacrifices that you've made on my behalf. Mum would have been proud of you. I would also like to thank to my beloved wife, Mariposa. Thank you for supporting me for everything, and especially I can't thank you enough for encouraging me throughout this experience. I would like to express my special appreciation and thanks to my advisor Professor Dr. Bruno Grassi, you have been a tremendous mentor for me. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. Your advice on both research as well as on my career have been invaluable. You are one of the smartest people I know. I hope that I could be as lively, enthusiastic, and energetic as you and to someday be able to command an audience as well as you can. I am also very grateful to Dr. Mauro Marzorati for his scientific advice and knowledge and many insightful discussions and suggestions. You are my primary resource for getting my science questions answered and were instrumental in helping me crank out this thesis, all in one "week". I would also like to thank my committee members, Professor Roberto Bottinelli and Professor Claudio Brancolini for letting my defense be an enjoyable moment, and for your brilliant comments and suggestions, thanks to you.

I would especially like to thank Dr. Lucia Morandi, physicians and nurses in the neuromuscular unit at Institute Carlo Besta. All of you have been fundamental to recruit patients and collected data for my Ph.D. thesis.

I am also very grateful to "Cuttigghiu" group: Giuseppe, Matthew, Letizia, Stefano, Lucrezia, Lorenzo e Fabio. I was lucky to be a part of this group and I know that I could always ask you for advice and opinions on lab related issues. A special thank goes to Lorenzo e Gaspare. I'll never forget the many wonderful lunches and fun activities we've done together, including that "spinach" experience. I will also not forget Alessandra (a nice and helpful person who has been pretty supportive during my research), Simona (a friendly colleague who has also helped me with the car selection), Michelle (always friendly and cheerful about everything) and Sara (you are the most rock'nd roll biologist that I have never met). I will forever be thankful to my former research advisors, Professor Claudio Marconi and Paolo Cerretelli. They have been helpful in providing advice many times during my career. I thank also to Professor Giovanna Rizzo, who encouraged me towards a more collective thinking.

If I have forgotten anyone, I apologize. I also thank my friends (too many to list here but you know who you are!) for providing support and friendship that I needed.