A multi-parametric protocol to study exercise intolerance in McArdle’s disease

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Introduction

McArdle’s disease, or glycogen storage disease type V (GSDV, OMIM #232600), is the most common metabolic myopathy of muscle carbohydrate metabolism, with a prevalence of 1 in 100000 individuals (1), caused by homozygous or compound heterozygous mutations in the PYGM gene on chromosome 11q13, which encodes muscle glycogen phosphorylase (2, 3). The myophosphorylase deficiency is responsible of the alteration of glycogen breakdown in muscle, which is an important fuel for the contraction of muscles, especially in prolonged exercise at high intensities.

The clinical symptoms of GSDV usually begin in young adulthood with exercise intolerance, exercise-induced muscle cramps, pain and recurrent episodes of myoglobinuria, which may lead to acute renal failure. Many patients experience the second wind phenomenon, characterized by an improved tolerance for aerobic exercise approximately after seven to eight minutes of motor activity (2, 3). The second wind phenomenon is determined by the increased availability of blood glucose and free fatty acids associated to an enhanced glucose uptake by muscle cells (2, 3).

Several dietary and pharmacological treatments have been tested to alleviate symptoms in this disease, but most of them failed to demonstrate a significant...
amelioration or were not well tolerated. The previous studies have often included a small number of patients; furthermore the clinical heterogeneity of GSDV might make it difficult to establish measurable primary outcomes (4, 5).

The aim of our study was to define a multi-parametric evaluation protocol in patients with GSDV in order to detect and quantify the impairment of both muscular energetic metabolism and motor performance. The protocol has been subsequently tested to investigate the effect of a carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine on exercise intolerance and motor skills in the same patients.

Material and methods

Patients recruitment

We enrolled 4 patients affected by GSDV (1 female, 3 males; age ranging from 19 to 58 years), including two brothers and two unrelated patients. All patients had experienced life-long exercise intolerance, repeated exercise-induced episodes of muscle cramps and occasional myoglobinuria. The diagnosis was confirmed by genetic and/or biochemical testing on muscle biopsy (Table 1). We also enrolled a female patient (patient 5, Table 1) reporting exercise intolerance that presented a partial myophosphorylase deficiency and was heterozygote for the mutation R50X in PYGM gene. A control group of 5 sex- and age-matched healthy subjects was included. Any subjects took medication at the time of the study. All patients and healthy subjects were informed of the risk and nature of the study and gave consent to participate.

Multi-parametric evaluation protocol

Pre-experimental preparations

Both patients and healthy subjects, following a free diet, were continuously monitored for three days with a metabolic holter SenseWear Armband. Patients and controls underwent to exercise protocol on a bicycle ergometer (described above). Afterwards the patients followed a carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine (6) for three days, during which they were monitored again with the armband. The composition of the carbohydrate-rich diet was 20% fat, 15% protein and 65% carbohydrate (vegetarian food: vegetables, fruits, pasta, rice, bread and low-fat cheese). The amount of the calories in the diet was adjusted to the subject’s weight, age and sex. The caloric intake was on average 2700 kcal for men and 2200 kcal for women. Each patient was instructed about the diet by a nutritionist. During the carbohydrate-rich diet patients also took 4 capsules/day of a food supplement called “CREATINE STRONG MATRIX 7”, containing creatine malate, creatine ethyl ester HCL, creatine alpha ketoglutarate, creatine orotate, creatine pyruvate, creatine citrate. As one capsule contains 1.22 g of creatine, the used daily dose of creatine resulted safe, according to previous reports (7, 8). Then, after the three days of diet, the patients repeated the test on the bicycle ergometer.

Metabolic Holter

Daily physical activities was objectively measured using a validated multisensory array, the BodyMedia SenseWear Armband (9). SenseWear Armband is a wearable device that utilizes a 2-axis accelerometer, heat flux sensor, galvanic skin response sensor (GSR), skin temperature sensor, and a near-body ambient temperature sensor to capture data leading to the calculation of energy expenditure (9). In this study we used Armband to measure: Basal Metabolic Rate (BMR), Total Energy Expendi-

| Patients | Age (years) | Sex  | BMI   | Myophosphorylase staining muscle biopsy | Genetic test PYGM gene          |
|----------|-------------|------|-------|---------------------------------------|---------------------------------|
| Patient 1* | 23          | Male | 26,78 | Absent                                | Homozygous mutations R50X       |
| Patient 2* | 19          | Male | 25,25 | Absent                                | Homozygous mutations R50X       |
| Patient 3 | 58          | Male | 30,07 | Absent                                | --                               |
| Patient 4 | 34          | Female | 24,09 | Absent                                | --                               |
| Patient 5 | 39          | Female | 19,15 | Reduced                               | Heterozygous mutations R50X     |

(*brothers)
ture (TEE), number of average METs (1 MET=1 Kcal/kg/hour; a normal healthy subject has a BMR of 1 MET, and positive or negative deviations from this value suggest respectively a hypermetabolic or a hypometabolic condition), number of steps, Physical Activity Duration (PAD), Active Energy Expenditure (AEE).

The device was worn by the enrolled subjects continuously for 72 hours during daily activities and during the exercise protocol.

Exercise test protocol.

All subjects were tested between 9 and 10 a.m; they had breakfast 2-2½ hours before exercise testing. They performed a cardiopulmonary exercise test (CPET) on a cycle ergometer at a constant workload of 50% of VO\textsubscript{2} max for 12 minutes, followed by an incremental test until exhaustion, using increments in workload of 15 watts every two minutes. The constant workload test was used to evaluate the occurrence of the second wind phenomenon, the heart rate and the rating of perceived exertion. The incremental test was used to determine the maximal oxidative capacity. The level of perceived exertion was scored every minute, using a visual analogue scale (Borge scale, 10). Cardiopulmonary, respiratory and metabolic responses to exercise were monitored continuously through the measurement of VO\textsubscript{2}, VCO\textsubscript{2}, RER, VE/VO\textsubscript{2}, VE/ VCO\textsubscript{2}, PETO\textsubscript{2}, PETCO\textsubscript{2}, HR, O\textsubscript{2} pulse (see the legend).

Blood samples were obtained at rest, exercise peak and recovery to analyse plasma lactate and oxidative stress biomarkers such as advanced oxidation proteins products (AOPP) and thiols (11, 12).

Furthermore, during exercise, a surface electromyography (sEMG) monitoring on quadriceps femoris muscle using wireless platform was performed, in order to analyze the sEMG signal during dynamic contractions and to study the muscular activity (13). sEMG technology is a non-invasive and non-painful analysis that allows information regarding the overall muscle function and condition collected from the surface of the skin. After the signal acquisition and filtering for reduction of artifacts, the energy values, expression of the motor units recruitment, for each 20 seconds after full wave rectification and smoothing using a low pas filter 2.4Hz (13) were extracted.

Statistical analysis

Differences between patients and controls, at baseline and after dietetic treatment, were assessed by a Student’s t-test; an analysis of variance was used to test whether significant changes in measured variables occurred with time. A p value < 0.05 was considered statistically significant.

Results

Metabolic Holter at basal conditions during a free dietary regimen

BMR resulted higher in controls then in patients. Also TEE was higher in controls then in patients, especially in terms of AEE. According to these data, the daily number of steps is on average 13253 in controls, whereas in patients is less then 10000, indicating a very low daily physical activity.

Even the average METs was higher in controls (1,6) then in patients (1,4) (Table 2).

Metabolic Holter during carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine

No significant difference between baseline and the three days diet treatment was observed in the patients group during daily activities.

Exercise protocol performed during a free dietary regimen

During the constant workload exercise on the bicycle ergometer, 3 patients experienced the characteristic second wind phenomenon with a peak heart rate of 143,3 bpm at 8 minute of exercise (Figure 1) and one patient was unable to complete the exercise because of the onset of cramps and fatigue. In the patient with partial myophosphorylase deficiency (heterozygous carrier, patient 5) the heart rate increased progressively during the exercise, as in healthy subjects. Heart rate was consistently lower in healthy subjects then in patients (p < 0.05), suggesting they were performing at a higher percentage of their exercise reserve. During the incremental workload, the patients managed to reach lower

|                  | Patients | Controls |
|------------------|----------|----------|
| BMR (cal)        | 1565     | 1565     |
| BMR (METs)       | 0.88     | 0.94     |
| TEE (cal)        | 2345     | 2683     |
| PAD (hours)      | 1.05     | 2.23     |
| AEE (cal)        | 292      | 605      |
| Steps number     | 7488     | 13253    |
| Sleeping duration (hours) | 6:35     | 6:17     |
| METs average     | 1.4      | 1.6      |

Table 2. Comparison of average values of holter metabolic monitoring between patients and healthy controls.
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maximal workload levels compared to healthy subjects, with a higher score of perceived exertion and higher levels of heart rate. The perceived exertion reflected the heart rate trend.

Both VO$_2$max and VCO$_2$max were significantly lower in patients (p < 0.05), according to the oxidative capacity impairment observed in McArdle’s disease. RER value was constant and < 1 in patients, while increased progressively to 1 value in controls, indicating the inability to use glycogen by muscle during sustained exercise, in McArdle’s disease.

The monitoring of the muscle activity by sEMG during constant workload and incremental exercise on the bicycle ergometer showed that the value of energy extracted from sEMG signal analysis was higher in patients than in healthy subjects (p < 0.03), suggesting they were performing the exercise at a higher percentage of their motor reserve.

Exercise protocol performed after the carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine

No significant difference between baseline and the three days dietary treatment was observed in the group of patients.

Blood lactate

- At basal conditions during a free dietary regimen.
  The typical “flat curve” was observed in the patients, as shown in Figure 2.

- After carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine.
  No significant difference between baseline and the results obtained after three days of carbohydrate-rich diet integrated with TCA intermediates and creatine was observed.

Oxidative stress biomarkers

- At basal conditions during a free dietary regimen.
  The analysis of blood oxidative stress biomarkers at exercise rest, peak and recovery showed significantly higher levels of thiols in patients than in healthy subjects, at basal condition and at peak of exercise (p<0.05) (Figure 3). Lower levels of AOPP in patients than in healthy subjects were also observed, although this difference did not reach a statistical significance.

- After carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine.
  No significant differences in oxidative stress biomarkers levels during exercise performed in basal conditions and after the three days dietary treatment were observed in the patients group.

Discussion

In this study we defined a multi-parametric evaluation protocol in patients with GSDV in order to characterize the impairment of the muscle metabolism and motor performances. Because a previous report observed a ben-
The deficit of oxidative metabolism in McArdle disease, it could be supposed that the higher levels of thiols detected in our patients could be due to the low flow of substrates through the TCA cycle which limits the oxidative capacity, oxygen consumption and oxidative phosphorylation in mitochondria and consequently the physiological production of reactive oxygen species (ROS) in basal condition and during the motor activities. The impairment of muscle mitochondrial respiration has been previously shown with $^{31}$P-MR spectroscopy (18). However these data should be confirmed in a larger cohort of patients. Anyway, it should be also consider that the duration of exercise effort was on average minor in patients then in healthy subjects and that the amount of ROS produced during exercise is proportional to its duration. Finally by the same protocol the effect of three days of carbohydrate-rich diet integrated with tricarboxylic acid cycle intermediate and creatine was investigated. In this respect, partially conflicting evidences on significant benefits from vary specific nutritional treatment in GSDV are reported in literature. It has been hypothesized that a carbohydrate-rich diet might improve exercise intolerance in GSDV patients by maintaining high glycogen stores in the liver. This hypothesis is supported by the key role of blood glucose for generating the second wind phenomenon, caused by an enhanced uptake and oxidation of glucose and, to a smaller extent, fatty acids (2). The mobilization of hepatic glucose is exaggerated during exercise in patients with GSDV because of a higher sympatho-adrenal response that is brought about by the initial energy crisis early in exercise. The glucose resulting from hepatic glycogenolysis results crucial for partially compensation of the blocked muscle glycogenolysis (14, 19). Previous single-case studies have also suggested that a protein-rich diet could be beneficial in GSDV patients (20, 21). In a previous cross-over open-design study (14) a carbohydrate-rich diet (20% fat, 15% protein, 65% carbohydrate) was proved to increase the maximal work capacity and exercise tolerance of submaximal workload in comparison with protein-rich diet (15% fat, 55% protein, 30% carbohydrate) in 7 GSDV patients. However, the authors suggested that other trials were needed to confirm the effect of carbohydrate-rich diets in GSDV, also by comparing with non-protein-rich diets and assessing the long-term effects (14).

In our study, the effect of a carbohydrate-rich diet on motor performance and exercise intolerance in 4 GSDV patients was analyzed by comparing the results obtained in basal conditions and after the diet, integrated with TCA intermediates and creatine, in order to improve oxidative capacity in these patients. In fact, the limited glycolysis in GSDV during exercise inevitably produces low concentrations of TCA cycle intermediates (22).
Using the same multi-parametric exercise protocol, we did not observed any differences between basal condition and three days diet; only one patient referred a mild subjective benefit in daily activities during the diet, with reduced fatigability. These results are apparently disagree with the ones described by Andersen and Vissing in 2008 (14), although the latter study reported a benefit of carbohydrate-rich diet only in comparison with a protein-rich one. Notably, several lines of evidence suggest that extra protein should not be helpful in McArdle disease. In fact, amino acids, the constituent of proteins, play a minor role in muscle energy metabolism during exercise, which is covered almost exclusively by fat from adipose tissue and by carbohydrates derived from hepatic and muscle glycogen stores (23). Therefore, we hypothesize that the absence of benefit of carbohydrate-rich diet observed in our study may be due to the comparison with a baseline condition and no with a protein enriched diet, that can be deleterious on motor performances in GSDV. It is also important to note that the Mediterranean diet, daily followed by our patients, is per se a carbohydrate-rich diet, with percentage of macronutrients very similar to the ones of the diet followed by patients during the three days of treatment and it is by itself beneficial in these patients.

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Legend

VO₂ = oxygen uptake
VCO₂ = CO₂ production
RER = respiratory exchange ratio
VE/VO₂ = ventilatory equivalents for O₂
VE/ VCO₂ = ventilatory equivalents for CO₂
PETO₂ = end-tidal O₂
PETCO₂ = end-tidal CO₂
HR = heart rate
O₂ pulse = Oxygen pulse (oxygen uptake per heartbeat at rest)

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