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 become available for patients with glycogen storage disease type II. Previous studies have demonstrated clinical efficacy of enzyme replacement therapy, however, data on physiological variables related to exercise tolerance are scarce. Four glycogen storage disease type II 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 O2 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 glycogen storage disease type II patients enzyme replacement therapy 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.

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1. 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 α-glucosidase (GAA) or acid maltase. The enzyme catalyzes the hydrolysis of α-1,4 and α-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 α-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- to 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 O2 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.

2. Materials 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 α-glucosidase (Myozyme® Genzyme Corporation, Cambridge, Mass) was administered intravenously at a dose of 20 mg/kg every 2 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 1 s (FEV1), 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 min, 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 s of each load. The highest values reached by the patient were taken as “peak” values. Pulmonary ventilation ($V_E$, expressed in BTPS), O2 uptake ($V_{O2}$), and CO2 output ($V_{CO2}$), both expressed in STPD, were determined breath-by-breath by a metabolic cart (SensorMedics Vmax 29c; The Netherlands). Expiratory flow measurements were performed by a mass flow sensor (hot wire anemometer), calibrated before each experiment by a 3-L syringe at three different flow rates. Tidal volume and $V_E$ were calculated by integration of the flow tracings recorded at the mouth of the subject. $V_{O2}$ and $V_{CO2}$ were determined by continuously monitoring partial pressures of oxygen (PO2) and carbon dioxide (PCO2) 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 O2 and CO2 analyzers was performed before each experiment by utilizing gas mixtures of known composition. Gas exchange ratio (R) was calculated as $V_{CO2}/V_{O2}$. 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 ($\Delta[{deoxy(Hb + Mb)}]$), with respect to an initial value arbitrarily set equal to zero, were calculated and expressed in arbitrary units. $\Delta[{deoxy(Hb + Mb)}]$ was taken as an oxygenation index, since this variable is relatively insensitive to changes in blood volume [12]. $\Delta[{deoxy(Hb + Mb)}]$ 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 $\Delta[{deoxy(Hb + Mb)}]$ increase reached a plateau.
Results were expressed as mean values ± standard deviation (x ± SD). The statistical significance of differences between means was checked by paired Students’ t-test. The level of significance was set at $P < 0.05$.

3. 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 min) of exercise on a stationary bicycle.

One patient did not perform the spirometry tests. Data obtained in the other 3 patients are shown in Fig. 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 $\Delta$[deoxygenated Hb + Mb]] are shown in Fig. 2 (also for $\Delta$[deoxygenated 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. ml O$_2$ kg$^{-1}$ 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 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 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 $\Delta$[deoxygenated Hb + Mb]] peak, taken as an estimate of skeletal muscle fractional O$_2$ extraction (for details see [12]).

4. 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 non-significant 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 almost 2–3% in the percentage of predicted FVC [9]).

ERT increased peak exercise capacity and exercise tolerance. Significantly higher values of $V_{O2}$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 $V_{O2}$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 $V_{O2}$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, \(\Delta[\text{deoxy(Hb + Mb)}]\) 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 O2 uptake, cardiac output and skeletal muscle oxygenation, can play in the assessment and follow-up of late onset GSDII patients.

5. Conflict of interest

None.

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