

Enzyme Replacement Therapy in Late-Onset Pompe's Disease: A Three-Year Follow-up

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Pompe's disease is an autosomal recessive myopathy. The characteristic lysosomal storage of glycogen is caused by acid α -glucosidase deficiency. Patients with late-onset Pompe's disease present with progressive muscle weakness also affecting pulmonary function. In search of a treatment, we investigated the feasibility of enzyme replacement therapy with recombinant human α -glucosidase from rabbit milk. Three patients (aged 11, 16, and 32 years) were enrolled in the study. They were all wheelchair-bound and two of them were ventilator dependent with a history of deteriorating pulmonary function. After 3 years of treatment with weekly infusions of α -glucosidase, the patients had stabilized pulmonary function and reported less fatigue. The youngest and least affected patient showed an impressive improvement of skeletal muscle strength and function. After 72 weeks of treatment, he could walk without support and finally abandoned his wheelchair. Our findings demonstrate that recombinant human α -glucosidase from rabbit milk has a therapeutic effect in late-onset Pompe's disease. There is good reason to continue the development of enzyme replacement therapy for Pompe's disease and to explore further the production of human therapeutic proteins in the milk of mammals.

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Pompe's disease or glycogen storage disease type II is an inherited myopathy characterized by lysosomal accumulation of glycogen and caused by acid α -glucosidase deficiency. Differences in the age of onset and rate of disease progression distinguish infantile from late-onset subtypes.^{1,2}

Infants with classic infantile Pompe's disease manifest feeding difficulties, generalized muscle weakness, cardiomyopathy, and respiratory insufficiency. They have a median life span of 6 to 8 months and usually die because of cardiorespiratory failure.³

Late-onset Pompe's disease presents as a proximal myopathy with symptoms restricted to skeletal muscle. Limb-girdle weakness is often the first sign and may lead to scoliosis. Most patients become wheelchair dependent and may require artificial ventilation later in life.^{1,2}

Enzyme replacement therapy (ERT) is currently under investigation as treatment for Pompe's disease. This

therapeutic approach aims to supplement the deficiency of acid α -glucosidase by intravenous administration of highly purified enzyme, finding its way to the lysosomes via endocytosis.^{4–6} The same type of treatment has been utilized in other lysosomal storage disorders, whereby recombinant human enzymes are used and produced in genetically modified animal or human cells.^{7–10} The first clinically applicable recombinant human α -glucosidase became available through production in the milk of transgenic rabbits. After successful completion of preclinical investigations, we started clinical studies with this enzyme in early 1999.^{11–14} The first pilot study included four patients with classic infantile Pompe's disease. The procedure appeared to be safe, and positive effects were seen after 36 weeks of treatment.^{5,15} Currently, three of the four patients are still alive at an age of 5.5 years.⁶ Several other studies in infants were started with recombinant human

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α -glucosidase from Chinese hamster ovary (CHO) cells.¹⁶

We expanded our study to include subjects with late-onset Pompe's disease because they represent the largest group of patients. This is the first report to our knowledge of three patients with juvenile Pompe's disease, who have received weekly infusions of recombinant human α -glucosidase from rabbit milk over a 3-year period.

Patients and Methods

Study Design

The study was conducted as a single-center, open-label pilot study and approved by the institutional review board of the Erasmus MC. Written informed consent was obtained from the patients and the parents, if required. The study objective was to evaluate safety and efficacy of recombinant human α -glucosidase from rabbit milk (rhAGLU).

Inclusion Criteria

Clinical and laboratory findings had to be consistent with late-onset Pompe's disease. The diagnosis had to be established before the age of 15 years and confirmed by acid α -glucosidase deficiency and lysosomal glycogen storage in an open-muscle biopsy. Patients had to be older than 4 and younger than 35 years at inclusion. Developmental delays not explained by Pompe's disease, allergies, and other conditions that potentially could interfere with the evaluation of the study objectives were exclusion criteria.

Treatment

RhAGLU was provided by Pharming-Genzyme LLC (Leiden, The Netherlands) (Cambridge, MA). Enzyme purification and characterization was performed as previously described.^{13,14} The enzyme was administered intravenously as a 1 to 2mg/ml solution in saline with 5% glucose and 0.1% human serum albumin, initially in single weekly doses of 10mg/kg, and later 20mg/kg with a transition period of 15mg/kg (Fig 1).

Muscle Biopsy

Open muscle biopsies were taken at baseline and 12 and 24 weeks after start of treatment with 10mg/kg/week, and minimally 12 weeks after increasing the dose to 20mg/kg/week (see Fig 1). The biopsies were performed 24 hours after the rhAGLU infusion. Tissue specimens for measurement of acid α -glucosidase activity and histology were prepared as described.^{6,17,18}

Assessments

The pulmonary function (EVC/FEV1) was measured with spirometry. Historical data were used for comparison. Muscle strength was measured with the Citec handheld dynamometer (HHD)^{19,20} by trained physical therapists and with the Medical Research Council (MRC) score by neurologists.²¹ The scores given to each muscle group were added to obtain a total score for upper, lower, and total body. The muscle groups tested were neck flexion, neck extension, shoulder abduction, elbow flexion and extension, wrist exten-

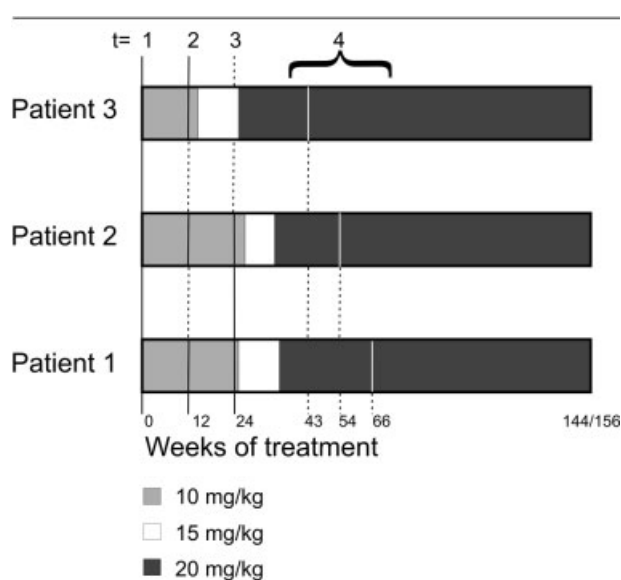


Fig 1. Overview of the dosing regimen and the time of biopsy for each patient. Biopsies were taken before treatment ($t = 1$), after 12 weeks ($t = 2$), and after 24 weeks ($t = 3$) with a dose of 10mg/kg/week and after 12 weeks of 20mg/kg/week ($t = 4$). The total treatment duration of Patients 1 and 2 was 156 weeks and 144 weeks for Patient 3.

sion, jaw chuck (MRC only), hip flexion and abduction, knee extension and flexion, and ankle dorsiflexion and plantar flexion. The maximum MRC score is 114 (normal strength of all muscles), and the minimum score is 0 (complete paralysis).

Muscle function was evaluated using the Gross Motor Function Measure (GMFM) and via timed tests (10-meter walk, the nine-hole peg, and rising from a chair and from the floor).²² Disability was evaluated with the Pediatric Evaluation of Disability Inventory (PEDI).²³ Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), creatine kinase (CK), and lactate dehydrogenase (LDH) were measured according to routine procedures.³ Patient interviews and physical examinations documented clinical follow-up.

Statistical Analysis

The slopes of fitted curves were calculated using least-squares regression. In the evaluation of CK, outcomes were logarithmically transformed. Piece-wise linear regression ("broken-stick" method) was applied to visualize and calculate changes in pulmonary function. Correlation coefficients given are Spearman's. p values less than or equal to 0.05 were considered significant.

Patients

Table 1 summarizes the clinical histories. Patients 1 (16-year-old girl) and 2 (32-year-old man) were in a far advanced stage of the disease. They were wheelchair-bound and partially (Patient 1) or fully (Patient 2) dependent on artificial ventilation. The clinical condition of Patient 1 was complicated by a progressive scoliosis, which had started at the age of 13 years. It progressed rapidly from a 30-degree right tho-

Table 1. Characteristics of the Patients at Start of the Treatment

Characteristic	Patient 1	Patient 2	Patient 3
Age at inclusion (yr)	16	32	11
First symptom (age)	Difficulty climbing stairs (10 yr)	Difficulty lifting head during sports (7 yr)	Feeding difficulties (6 mo)
Age at diagnosis (yr)	11	10	2.5
Developmental milestones	Normal	Delayed	Delayed
Early motor development	Clumsy	Clumsy	Clumsy
Age walking	14 mo	2 yr	2.5 yr
Frequent airway infections	Yes	Yes	No
Ventilator dependency (age)	12 yr	15 yr	—
Ventilator use	18 hr/day	24 hr/day	—
Fatigue	Yes	Yes	Yes
Use of wheelchair since (age, yr)	16	22	9
Scoliosis/correction (age, yr)	13	14/15	—
Contractures	Yes	Yes	Yes
Genotype	[271G>A + 877G>A] “unknown”	IVS1-13t>g 1548G>A	IVS1-13t>g 525delT

racic curve at age 14 years to a 60-degree right thoracic curve and a left lumbar curve of 74 degrees at the time of initiation of the study. Patient 3 was moderately affected. He had a normal pulmonary function at initiation of treatment but used a wheelchair since age of 2 years.

All three patients had mutations (see Table 1) and enzyme deficiencies (Table 2, column $t = 1$) consistent with the diagnosis of late-onset Pompe's disease.

Results

This report describes a 3-year period wherein three patients with juvenile Pompe's disease received weekly infusions with recombinant human α -glucosidase from rabbit milk. Two patients did not experience an infusion-related reaction. The third patient had a 3-month period of mild and transient skin reactions and received premedication with corticosteroids, antihistamines, and cromoglycate.

Muscle Strength and Function

The least affected patient (Patient 3) showed a drastic gain of muscle strength and function. The total HHD

score increased from 392 to 4,684 Newton ($r = 0.97$; $p < 0.001$) and a total MRC sum score from 74 to 114 (maximum score) ($r = 0.92$ $p < 0.001$) over 144 weeks of treatment (Fig 2). The GMFM score improved from 56.5 to 100% ($r = 0.99$ $p < 0.001$).

At the start of treatment, Patient 3 was wheelchair-bound and could not stand or walk (Fig 3A). After 72 weeks of therapy, he could rise with difficulty from a chair using the Gowers' maneuver and managed 10 steps on tiptoes. His muscle strength and function continued to improve after an Achilles tendon release procedure at 75 weeks of treatment. He performed the 10-meter walk test in 41 seconds in week 84, and 24 weeks later in only 3 seconds (see Fig 3B). Further improvement was recorded by cycling against resistance, from 140 Watt after 108 weeks to 180 Watt (within the 10th percentile) after 132 weeks.

Patient 2 also showed a significant increase of muscle strength (HHD $r = 0.72$ and MRC $r = 0.87$, $p < 0.001$; see Fig 2). At the start of treatment, he was virtually tetraplegic, but during treatment his leg, arm,

Table 2. α -Glucosidase Activity and Glycogen Content in the Muscle Biopsies

Patient No.	$t = 1$	$t = 2$ (12 \times 10mg)	$t = 3$ (24 \times 10mg)	$t = 4$ (12 \times 20mg)
α -Glucosidase activity				
1	2.6	3.1	4.9	3.0
2	1.3	5.7	7.9	8.4
3	0.8	1.1	ND	2.9
Reference range		8–40nmol 4MU/h/mg protein		
Late-onset patients		0.6–2.6nmol/h/mg		
Glycogen content				
1	1,045	1,415	1,445	745
2	276	503	424	232
3	141	99		45
Reference range		30–180 μ g glycogen/mg protein		

ND = not determined.

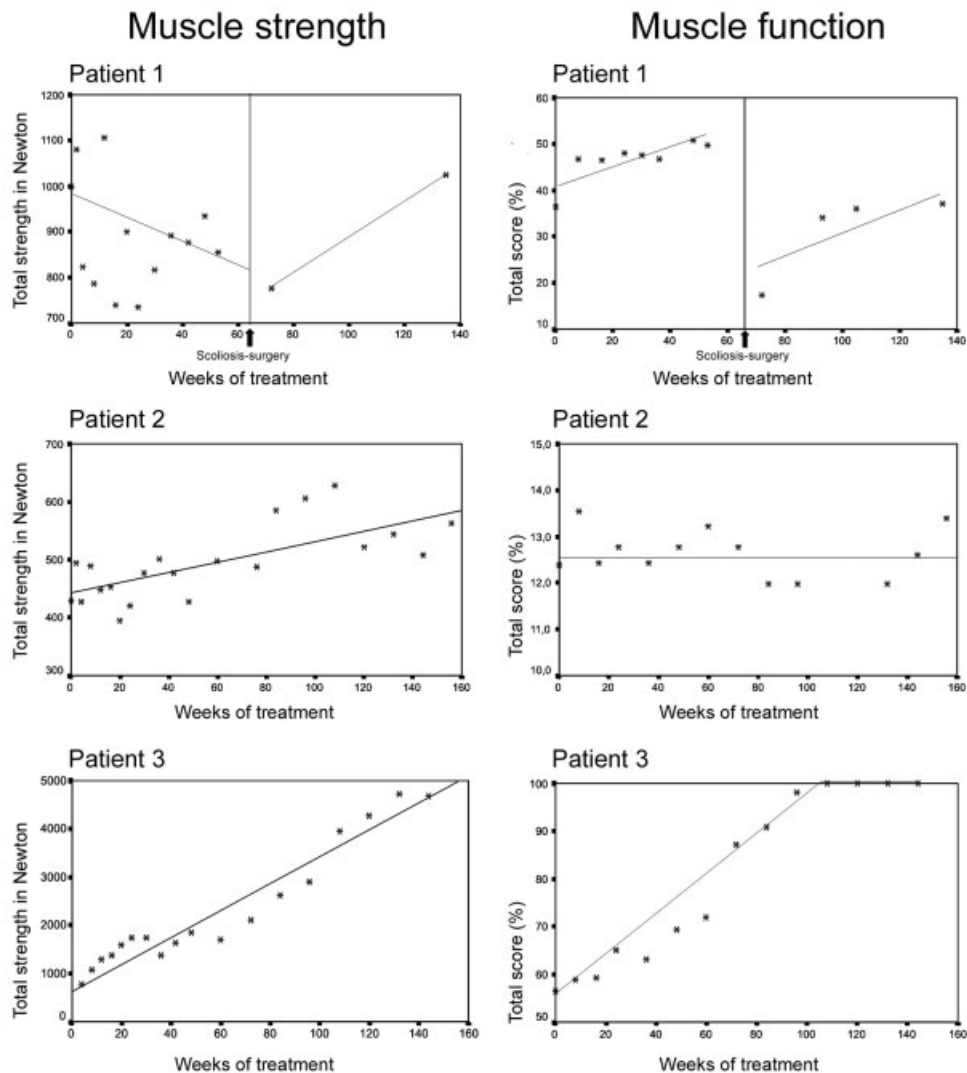


Fig 2. Effects of treatment on muscle strength and function. The muscle strength was measured with handheld dynamometry (left panels); the Gross Motor Function Measure was used as a measure for muscle function (right panels).

and neck muscles became a little bit stronger. This led to higher scores for self-care items in the PEDI questionnaire (dressing and washing).

Patient 1 lost muscle strength of the lower body during the first 53 weeks of treatment, mainly because of a progressive scoliosis. The right thoracic curve progressed from 60 to 68 degrees and the left lumbar curve from 74 to 90 degrees. Lumbar pain and the appearance of a Babinski reflex accompanied this. She lost the ability to walk without assistance ($r = -0.84$, $p = 0.010$), but kneeling, crawling, and sitting improved ($r = 0.90$, $p = 0.002$), leading to a significantly higher total GMFM score ($r = 0.86$, $p = 0.007$; see Fig 2).

Between week 64 and 66, the right thoracic and left lumbar curve were surgically corrected to 28 degrees. Thereafter, the patient regained strength and function, more so in the upper than in the lower limbs. She

learned to walk between parallel bars, but requires a wheelchair in daily functioning. Scores on self-care items of the PEDI questionnaire improved gradually (washing and dressing).

Pulmonary Function

Both Patients 1 and 2 had a significant decline of vital capacity (VC) in the 6 to 9 years before the start of treatment, down to 14 and 9% of normal, respectively (Patient 1, $r = -0.99$, $p < 0.001$; Patient 2, $r = -0.98$, $p = 0.021$). Using the “broken-stick” method, the slope of VC changed significantly after the start of treatment for both patients (Patient 1, $p = 0.002$; Patient 2, $p < 0.001$; Fig 4). The VC of Patient 2 increased significantly to 16% ($r = 0.58$, $p = 0.024$).

Patient 3 had a normal age-related increase of VC ($p < 0.001$) during treatment, both in supine and sitting position (values between 87 and 98% of normal),

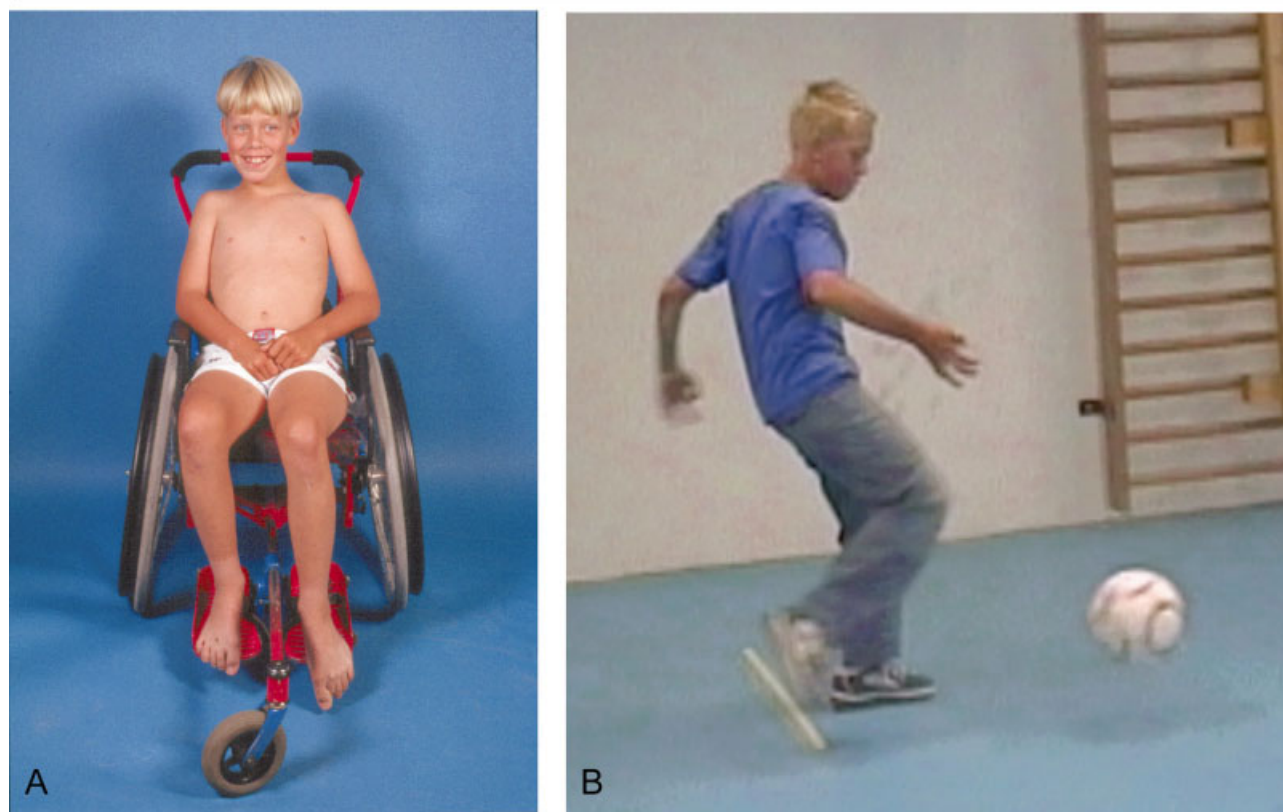


Fig 3. Patient 3 before treatment (A) and after 100 weeks of treatment (B).

whereas his VC remained constant in the 3 years preceding treatment. The change was significant (sitting $p = 0.023$, supine $p < 0.001$).

Quality of Life

All three patients gained energy and quality of life during treatment. Patients 1 and 2 needed less ventilation. Patient 1 resumed her education, started courses at a university, and participates in social life. Her ventilator need decreased from 18 to 10 hours per day. Patient 2 was frequently hospitalized before the start of treatment because of airway infections. During treatment, the infections usually resolved without antibiotics, and admissions were no longer required. Instead of being bedridden for most of the day (21 hours at start of treatment), he could stay up for 13 hours a day and go out. Telephone conversations became possible, as his speech improved.

Patient 3 was the best responder. He used to ride in a wheelchair in the 2 years preceding and the first 2 years after start of treatment. He can ride his bicycle for more than 25km and plays sports with friends. He now attends school for 4 days a week and receives his medication on the fifth day.

α -Glucosidase Uptake and Glycogen Degradation

Table 2 shows the α -glucosidase activities in skeletal muscle (see Fig 1 for time points). After 12 to 24

weeks of treatment with 10mg/kg, we measured a slight increase of α -glucosidase activity compared with baseline. To optimize the therapeutic effect, the rhA-GLU dose then was increased to 20mg/kg/week. Twelve weeks later, α -glucosidase activities were 2.9 to 8.4nmol MU/mg/hour and substantially above baseline (0.82–2.6nmol MU/mg/hour) but still below normal (8–40nmol MU/mg/hour). The glycogen content decreased slightly at the higher dose (see Table 2).

Routine Laboratory Results

The CK levels of all patients decreased significantly during treatment, particularly of Patient 1 (from 1,560 to 545 IU, $p < 0.001$; Fig 5). ALAT, ASAT, and LDH activities also decreased (not shown), and Patient 3 reached near reference values for his age after 144 weeks of treatment. Other biochemical parameters measured for safety reasons did not change during the treatment.

Muscle Morphology

Muscle sections of the quadriceps showed a lower periodic acid–Schiff staining intensity as a result of treatment. Periodic acid–Schiff–stained vacuoles had disappeared from the endothelium after 12 weeks of treatment with 10mg/kg and gradually also disappeared from the smooth muscle of the arteries and veins. Gly-

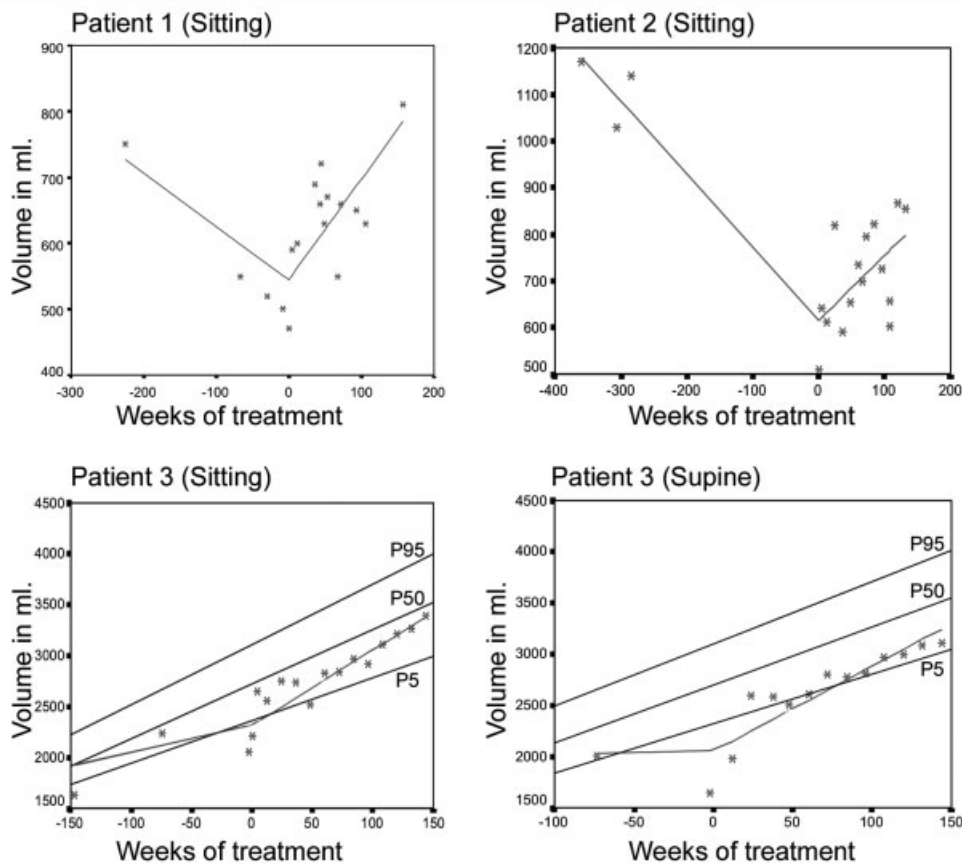


Fig 4. Expiratory vital capacity before and during treatment. The change after start of treatment was calculated with the broken-stick method.

cogen storage in peripheral nerves also was corrected. The muscle fibers remained variably affected in Patients 1 and 2 but regained a near normal morphology in Patient 3 after 43 weeks of treatment (not shown).

Discussion

This study shows for the first time to our knowledge that patients with late-onset Pompe's disease can benefit substantially from long-term intravenous administrations of recombinant human α -glucosidase from rabbit milk, like patients with infantile Pompe's disease.^{5,6}

As the risks accompanying a new form of therapy can overrule the benefits, we limited our study to three patients in different stages of the disease. As a consequence, the patients responded differently in pulmonary and muscle function tests so that the effects needed to be evaluated individually.

Efficacy

The effect of treatment was most significant in the least affected patient. He gained normal muscle strength and function, and his pulmonary function increased steadily according to his age. The two severely

affected patients benefited from the treatment mainly through a lower degree of disability and improvement of quality of life; however, they remained wheelchair-bound. Their pulmonary function stabilized. In parallel with these clinical accomplishments, a decrease of the CK, ALAT, ASAT, and LDH levels was recorded. We noticed that the best responding muscle groups were those that were actively used. The distal muscles responded better than the proximal. The same was observed in the infantile study group.¹⁸ We assume that this relates to the higher blood flow in active compared with resting muscle and to the percentage of muscle fibers with sufficiently preserved structure to actively capture and deliver the administered enzyme to the lysosomes. Moreover, muscle activity leads to an increase of insulin-like growth factor-1 and enhanced satellite cell proliferation, needed for muscle cell regeneration.²⁴

Safety

Producing recombinant human α -glucosidase in transgenic rabbits and extracting it from the milk results in a remarkably safe product for intravenous administration. The three patients tolerated the weekly infusions with the relatively high dose of 10 to 20mg/kg, gener-

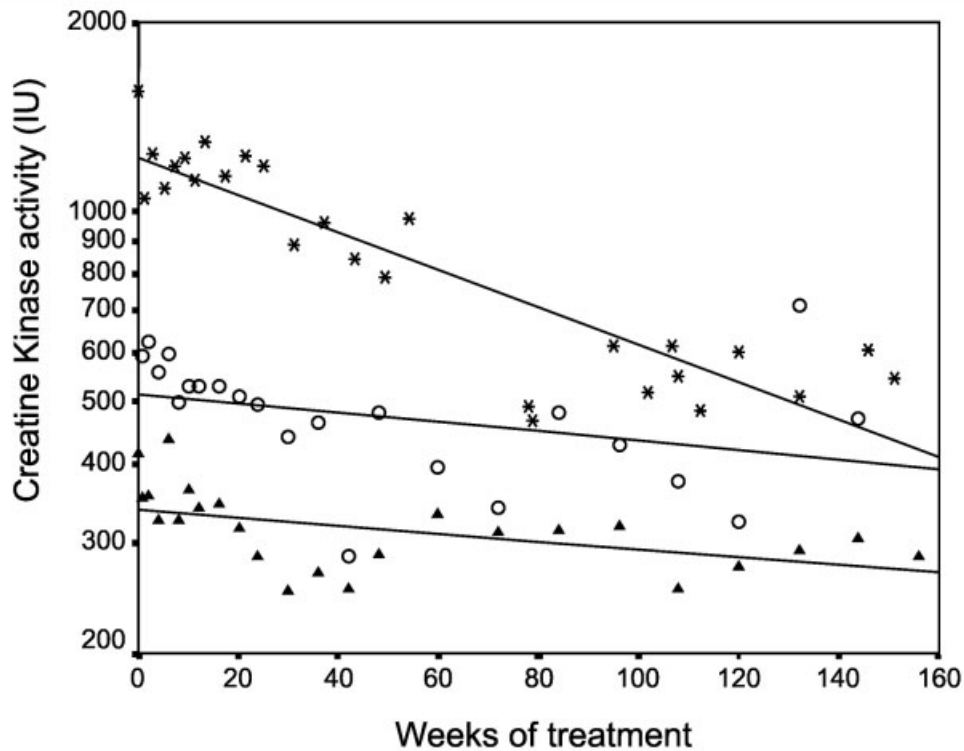


Fig 5. Effect of treatment on creatine kinase levels. (asterisks) Patient 1; (triangles) Patient 2; (circles) Patient 3.

ally without premedication. A similar tolerance was observed in the four infantile patients.^{5,6,15} Note that food and protein allergy was an exclusion criterion. Premedication frequently is used for the treatment of Fabry's disease with recombinant human enzymes from CHO or human cells in much lower doses.⁷⁻⁹

We did measure an IgG type of antibody response, despite the fact that all three patients had residual synthesis of endogenous α -glucosidase. The immune response did not interfere with the effect of treatment as reported in two patients with infantile Pompe's disease receiving recombinant human enzyme from CHO cells.¹⁶

Patient Selection and Clinical End Points

Our findings provide a solid basis for further development of enzyme replacement therapy for late-onset Pompe's disease. Improvement of muscle strength, muscle function, and vital capacity appear to be suitable end points for a future pivotal trial.

The MRC works better for measuring the strength of weak muscles than the HHD. Gain of muscle function is more relevant to the patient and can be measured reliably with the GMFM. If patients have sufficient mobility, timed tests can be added. The PEDI demonstrated to be a useful instrument to record subtle improvement of the patients, at the level of self-care and mobility.

For patient selection, our study illustrates that it is

desirable to work in future with a rather homogeneous group of moderately affected patients. Their age may differ, but it is important that they have similar residual muscle strength and function, and/or similar pulmonary function. Patients should be older than 6 years to perform the tests adequately.

We noticed in our studies that the process of recovery is slow and therefore recommend that a pivotal study should last for at least 1 year. It is advisable to collect historical data that can serve as inpatient control.

Decrease of plasma CK, reduction of muscle glycogen, and improvement of muscle morphology can be used as surrogate markers. However, muscle pathology can vary substantially between muscle bundles and fibers.¹⁸

Dosing and Production Capacity

The poor accessibility and the poor regenerative capacity of muscle tissue are obstacles for successful treatment of Pompe's disease. The circulating therapeutic enzyme must cross the capillary wall to reach the myocytes and can in this process be trapped by the lysosomal system of the endothelial and the interstitial cells. This is probably why the amount of enzyme needed to treat Pompe's disease exceeds the dose needed for treatment of Gaucher's disease and Fabry's disease.⁷⁻⁹ Our studies in infantile and late-onset Pompe's disease indicate that 20mg/kg is the minimal dose to target

α -glucosidase to the muscle and obtain a clinical effect.^{5,6,18} From this perspective, it is expected to be more effective to give high doses weekly or biweekly than low doses more frequently.

At a dose of 20mg/kg/week, the need of recombinant human α -glucosidase for an estimated 3,000 patients in the Western world is 150kg of enzyme formulation and approximately double this amount of crude preparation. With present-day technology, it is a great challenge to produce this large amount in CHO cells.²⁵ The economic burden of health care forces our society to search for alternative production platforms to keep up with the ever-increasing demand of sophisticated products.^{26,27} Our studies advocate more focus on transgenic technology because we have demonstrated that a product purified from milk of transgenic animals can be safe and effective for the treatment of human diseases.

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