NATURAL COURSE, EFFECTS OF ENZYME THERAPY AND PROGNOSTIC FACTORS IN ADULTS WITH POMPE DISEASE

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Erasmus ERASMUS UNIVERSITEIT ROTTERDAM

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**NATURAL COURSE, EFFECTS OF ENZYME THERAPY
AND PROGNOSTIC FACTORS IN ADULTS WITH POMPE DISEASE**

Natuurlijk beloop, effecten van enzymtherapie en prognostische factoren
bij volwassenen met de ziekte van Pompe

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CHAPTER 1

## General Introduction and Scope of the Thesis

The Erasmus MC University Medical Center has a longstanding tradition in fundamental and clinical research in the field of lysosomal storage diseases with a special focus on Pompe disease. The pioneering work of our research group has resulted in the successful implementation of enzyme replacement therapy (ERT) for patients with Pompe disease, turning it into the first treatable hereditary neuromuscular disorder. In 2007, the Center for Lysosomal and Metabolic diseases at Erasmus MC (formerly known as 'Pompe Center') was appointed by the Dutch health authority as the Center of Excellence for this particular disease. In this function, the center coordinates the care of all patients combined with basic and clinical research. The research performed at the center is denoted by its translational character, as basic research and clinical research go hand in hand.

The studies described in this thesis follow the tradition of combining patient care with translational research. This introductory chapter provides background information on Pompe disease with subjects like: clinical manifestations, natural disease course, diagnosis, pathogenesis, and treatment. The chapter ends with a description of the study aims and the content of the thesis.

POMPE DISEASE

Pompe disease derives its name from the Dutch pathologist Johannes Cassianus Pompe. In 1932 he published a case of a 7-month-old girl who was thought to have died from pneumonia, however the autopsy revealed an enormous heart and other enlarged organs.[1] Studying post mortem tissues using light microscopy, he found massive vacuolar storage of glycogen in most organs, called it 'generalized' glycogenosis and concluded that the disease could be due to a defect in the infant's glycogen metabolism. Accordingly, Pompe disease belongs to the *glycogenoses*, a group of metabolic disorders caused by deficiencies of one or another enzyme associated with glycogen metabolism. In 1952, a classification was made of the different glycogen storage diseases and Pompe disease was classified as *glycogen storage disease type II*.[2] About a decade later Hers and coworkers demonstrated deficiency of the lysosomal enzyme acid α-glucosidase in five infants with Pompe disease.[3][4] The enzyme cleaves both the α-1,4 as well as the α-1,6 glycosidic bonds at acid pH and thereby degrades the glucose polymer glycogen into glucose. Pompe disease became the first *lysosomal storage disorder* with a known enzymatic defect, and many other lysosomal enzyme deficiencies were subsequently discovered (**Figure 1**).[5] The discovery of the responsible deficient enzyme has given rise to the denominations *acid α-glucosidase deficiency* or *acid maltase deficiency*. In the following years, acid α-glucosidase deficiency was also identified as the cause of slowly progressive muscular weakness in children and adults, and therefore is one of the *neuromuscular diseases*.[6-8] Hence, the clinical spectrum of Pompe expanded from infants with severe muscle weak-
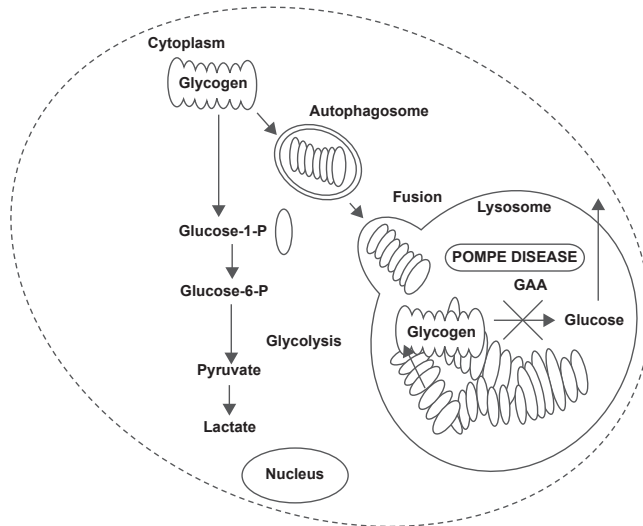
Figure 1 Pathways of glycogen metabolism and effect of GAA deficiency
Glycogen, a polymer of glucose, is transported to the lysosomes by autophagy and is used to store energy within the cells. When energy is needed, the intra-lysosomal glycogen is degraded into glucose by acid α-glucosidase (GAA). In case the enzyme is absent or deficient, glycogen accumulates in the lysosomes, which increase, swell and finally rupture. This causes cellular damage and finally loss of function. This figure was adapted from the publication by Raben *et al.* entitled 'Autophagy and mitochondria in Pompe disease: nothing is so new as what has long been forgotten' in the *American Journal of Medical Genetics* 2012, by permission of John Wiley and Sons.

ness and cardiomegaly to patients with onset of slowly increasing muscle weakness in late adulthood. In children and adults Pompe disease usually manifests itself as a limb-girdle myopathy, as pathological changes are most prominent in the skeletal muscles of the trunk and proximal limbs.[9][10]

There are over 600 hundred different neuromuscular diseases. Often it concerns genetic conditions having a low prevalence.[11] Nevertheless, it is estimated that in total about 200.000 – 400.000 people in the Netherlands have a neuromuscular disease.[12] For Pompe disease, worldwide estimates vary from one in 40.000 to one in 300.000 live births.[13-16] In the Netherlands, the overall incidence is 1 in 40.000 live births, stratified for phenotype the classic infantile form accounts for a predicted frequency of 1 in 138.000 and the childhood and adult form for a predicted frequency of 1 in 57.000.[14] Due to its low prevalence and severe nature, Pompe disease meets the criteria for classification as an *orphan disease*.[17]

From 1999 on, more patients have been referred to our center, as it was designated as the Center of Excellence for Pompe disease in the Netherlands. To date, we know of 26

patients with the classic infantile phenotype and of 135 patients with non-classic Pompe disease, 22 of whom were diagnosed in childhood and 113 in adulthood.

CLINICAL SPECTRUM, MANIFESTATIONS AND DISEASE COURSE

Clinical spectrum

The first reported cases of Pompe disease were infants who died of cardiorespiratory failure within the first year of life.[1][18][19] With increasing knowledge of the symptomatology of the disease, the clinical spectrum has expanded and actually comprises patients with first symptoms at any age and widely differing rates of disease progression.[10][20-23] All phenotypes together form a continuous clinical spectrum (**Figure 2**).[24] The clinically severest and rapid progressive classic infantile phenotype is situated on the one end of the spectrum. It is characterized by onset of symptoms within the first six months of life and by a hypertrophic cardiomyopathy.[25][26] The rest of the spectrum encompasses more slowly progressive forms with onset of symptoms in childhood or adulthood with only rarely, or no signs of cardiac involvement.[27-30] There is no consensus among experts on the nomenclature used for the different clinical subtypes of Pompe disease. Recently, the following nomenclature was proposed, which uses a mixture of old and new terminology and is practical for the use of reporting data: 1) classic infantile Pompe disease as defined above; 2) childhood Pompe disease for patients lacking the hypertrophic cardiomyopathy and possessing residual enzyme activity and with onset of symptoms from birth till adolescence; and 3) adult Pompe disease for patients similar as described in 2), but with onset of symptoms from adolescence to late adulthood.[24]
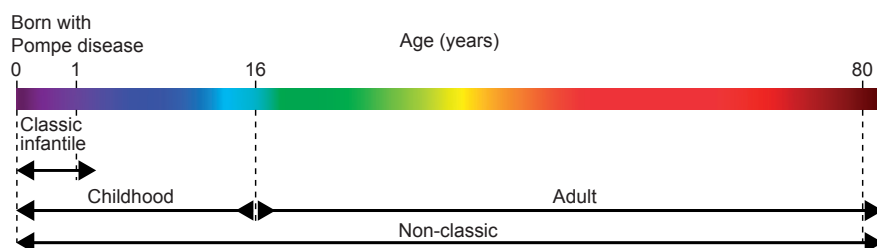
Figure 2 The clinical spectrum of Pompe disease
The figure was adapted from the publication written by Güngör and Reuser entitled 'How to describe the clinical spectrum in Pompe disease?' in the *American Journal of Medical Genetics* 2013, by permission of John Wiley and Sons.

Clinical manifestations and natural course in classic infantile Pompe disease

Patients with classic infantile Pompe disease almost always present with obvious symptoms within the first three months after birth. The symptoms consist of feeding difficulties, generalized muscle hypotonia (floppy infant), muscular weakness, failure to thrive and respiratory insufficiency predisposing for respiratory infections.[25 26] A minority of infants suffer shortly after birth from cardiac failure or cardiac arrhythmias. Due to rapidly progressive muscular weakness, the motor development is evidently delayed or even absent, patients do not learn to roll over or sit. Other symptoms and findings are macroglossia, moderate hepato(spleno)megaly, and hearing deficits due to the accumulation of glycogen in the sensory cells of the cochlea.[31] Untreated patients decease on average between six and seven months old due to cardiorespiratory failure.[25 26]

Clinical manifestations and natural course in childhood and adult Pompe disease

The initial symptoms in children and adults with Pompe disease can appear at any age from early infancy to late adulthood (range first to seventh decade) and comprise difficulties with running, performing sports, walking, climbing stairs or standing up from a chair.[20 21 28 32-37] The disease develops as a slowly progressive symmetrical limb-girdle myopathy without clinical signs of cardiac involvement. The disease also affects the respiratory muscles and diaphragm. In a cross-sectional study in the Dutch population about three quarter of the patients had some degree of pulmonary dysfunction and 38% had obvious diaphragmatic weakness (defined as an inability to endure testing in supine position, a large drop in VC upon changing posture (>25%), or decreased MIP).[38] About 10% of patients experienced presenting symptoms attributable to respiratory dysfunction, such as shortness of breath, disrupted sleep, or morning headaches. In 2% of the patients, the development of acute respiratory failure leads to the diagnosis of Pompe disease.[33 36 39] Fatigue was mentioned by a quarter of the patients as one of the earliest symptoms, and 76-85% of the patients report disproportional levels of fatigue.[32 40 41] Pain, mainly experienced in the legs, can also be an initial symptoms and is reported during the course of disease by 33-36% of the patients.[28 32 42]

Neurological examination often reveals less well known features of Pompe disease, which are sometimes symptomatic of other neuromuscular diseases, e.g. asymmetric ptosis,[43-46] bulbar weakness,[43 47 48] scoliosis,[48-50] and severe scapular winging.[27 48] Less frequently encountered features of the disease are macroglossia [51-53] and rigid spine.[54-56] It was shown in our cohort that these occurred in a considerable number of patients.[10]

The natural history is characterized by gradual progression of muscle weakness that in the majority (60-70%) of patients leads to wheelchair and ventilator dependency.[21 36] In general, the degree of skeletal muscle involvement is associated with the degree of pulmonary dysfunction, but there are patients with fairly limited motor problems who

are dependent on invasive ventilation.[32 34 38] The severity of disease usually relates to the disease duration, but the pace of decline may vary among patients, even among affected siblings.[23 33 57 58] About a quarter of the children that are diagnosed under the age of 15 years seem to have a relative severe and fast progressive form of Pompe disease.[21]

The life expectancy of children and adults with Pompe disease is shorter than the average normal for the general population.[59] Respiratory failure, sometimes associated with pneumonia and complications of immobility are often the cause of death.[36 60]

DIAGNOSIS

Differential diagnosis

Because this thesis has its focus on patients with non-classic forms of Pompe disease, the differential diagnosis of classic-infantile Pompe disease will not be discussed in this paragraph.

Most often the initial symptoms in non-classic Pompe disease are related to limb-girdle weakness. This gives rise to an extensive differential diagnosis, as a lot of defects in the peripheral nervous system can cause limb-girdle weakness: examples are defects in cell bodies located in the spinal cord (anterior horns cells), the nerve roots and nerve plexus, peripheral nerves, neuromuscular junction and the skeletal muscles (**Figure 3**). Alternative diagnoses causing these defects are for instance hereditary limb-girdle muscular dystrophies, myotonic dystrophy type II (PROMM) or other metabolic myopathies. **Table I** lists examples of diseases that are included in the differential diagnosis of childhood and adult onset Pompe disease. For instance, besides limb-girdle weakness, ptosis, bulbar weakness, scoliosis, macroglossia, rigid spine, lumbar lordosis, and severe scapular winging, are among the clinical features that Pompe disease shares with other neuromuscular diseases. With regard to ptosis, the differential diagnosis can be narrowed down to disorders like myasthenia gravis, oculopharyngeal muscular dystrophy (OPMD), myotonic dystrophy (DM) type 1 and mitochondrial myopathy; with regard to *bulbar weakness*, to Kennedy disease, myasthenia gravis, mitochondrial myopathy, OPMD, and DM type I; with regard to *macroglossia*, to Becker muscular dystrophy, amyloid myopathy in primary amyloidosis, and hypothyroidism; with regard to *rigid spine*, to rigid spinal muscular dystrophy (selonopathy-related myopathy / selenoprotein N1-related myopathy [SEPN1RM]), or rigid spine syndrome, but, rigid spine is also seen in different stages of various other myopathies;[61] with regard to *scoliosis*, to FSHD, polymyositis, dermatomyositis, sporadic inclusion body myositis (sIBM), OPMD, dystrophinopathy; and with respect to *scapular winging*, especially to facioscapulohumeral dystrophy (FSHD), and also to limb-girdle muscular dystrophy (LGMD) 2A and spinal accessory nerve lesions.[11]

Location in the PNS **Neuromuscular disease**

Motor neuron ----

Spinal Muscular
Atrophy

Nerve axon

Polyneuropathy

Neuromuscular
junction

Myasthenia Gravis

Muscle

Myopathy/
Muscular dystrophy
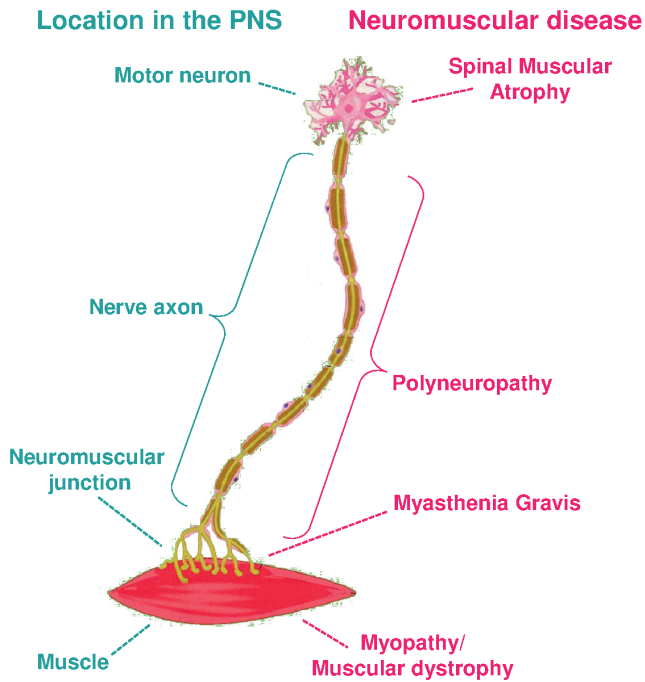
Figure 3 Neuromuscular diseases and the lower motor neuron
The lower motor neuron (anterior horn cells) receives input from the central nervous system to initiate contraction in the skeletal muscles by innervating several muscle fibers. The dendrites at one end of the nerve cell carry the signal (action potential) toward the cell body of the neuron via the neuron axon onto the axon terminals. Subsequently, acetylcholine, a small molecule excitatory neurotransmitter, diffuses through the synapse and binds nicotinic acetylcholine receptors on the plasma membrane of the muscle fiber, also known as the sarcolemma. The binding of acetylcholine to the receptor can depolarize the muscle fiber, causing a cascade that eventually results in muscle contraction. For several neuromuscular disorders, the location is indicated where the pathologic mechanisms affect the peripheral nervous system (PNS). The figure was adapted from the publication written by N.C. Notermans and P.A. van Doorn entitled 'Polyneuropathie: diagnostiek en beleid', in the *Nederlands Tijdschrift voor Geneeskunde* 1997, by permission of Bohn Stafleu van Loghum.

Table I Differential diagnosis of childhood and adult Pompe disease [a]

Toxic/endocrine causes
- Endocrinological disorders, e.g. hypothyroidism
- Myotoxic medication, e.g. statins, corticosteroids, interferon
- Toxic myopathy, e.g. red yeast rice, alcohol

Myopathies and muscular dystrophies
- Duchenne muscular dystrophy
- Becker muscular dystrophy
- Limb-Girdle Muscular Dystrophies (LGMD type 1 and 2; autosomal dominant and recessive)
- Emery-Dreifuss muscular dystrophy
- FacioScapuloHumeral Dystrophy (FSHD)
- OculoPharyngeal Muscular Dystrophy (OPMD)
- Myotonic Dystrophy (DM) type 1 and DM type 2 (PROMM)
- Glycogen Storage Diseases (GSDs)   - GSD type V / muscle phosphorylase deficiency / McArdle disease   - GSD type VII / muscle phosphofructokinase deficiency / Tauri disease
- Mitochondrial myopathies
- Amyloidosis
- Rigid spine syndrome
- Bethlem's myopathy
- Myofibrillar myopathy
- Myositis   - Dermatomyositis   - Polymyositis   - Sporadic Inclusion Body Myositis (sIBM)

Disorders of the neuromuscular junction
- Myasthenia Gravis (MG)
- Lambert-Eaton Myasthenic Syndrome (LEMS)

Disorders of the spinal cord
- Spinal Muscular Atrophy (SMA) type II, III and IV
- Kennedy disease (spinal and bulbar muscular atrophy)

[a] *The presented differential diagnosis does not pretend to be entirely complete*

Diagnostic process

In children and adults with limb-girdle muscular weakness the diagnostic guideline of the Dutch Neuromuscular Research Center ISNO (Interuniversitair Steunpunt Neuromusculair Onderzoek) entitled 'diagnosis of limb-girdle weakness' is very helpful for structuring the diagnostic workup.[62] The guideline recommends performing acid α-glucosidase testing in all patient with limb-girdle weakness of whom the family history is not indicative of another neuromuscular disease given that neurogenic, endocrinological and iatrogenic causes are unlikely or ruled out. This prominent position within the diagnostic work-up of adult patients with limb-girdle weakness can be explained by the fact that it is relatively easy to diagnose by enzymatic and DNA testing and also

because Pompe disease currently is the only inherited neuromuscular disorder for which a disease-specific treatment is available.

Enzymatic and molecular diagnosis

When a diagnosis of Pompe disease is suspected, first an enzyme assay in leucocytes is performed, as this is a rapid and decisive way for making the diagnosis. This assay uses either the natural substrate glycogen or the artificial substrate 4-methylumbellifery-alpha-D-glucopyraniside(4-MU) for measuring the acid α-glucosidase activity. In both cases the addition of acarbose to the incubation mixture is mandatory to eliminate the activity of glucoamylase.[63] The acid α-glucosidase activity as measured in cultured skin fibroblasts obtained by skin biopsy is the most reliable assay, but it takes more time. The assay is suitable for accurately measuring the level of enzyme activity and in most cases distinguishes patients with classic infantile Pompe disease from those with less progressive clinical phenotypes (*Reuser* Expert Opinion).

The test can also be performed using dried bloodspots, but requires more than other tests confirmation by alternative assay procedures.[35] The dried blood spot assay has its greatest value when used for screening purposes in patient populations at risk for Pompe disease or for neonatal screening.[64-66] Just as in dried blood spots, the quantification of glycogen-filled vacuoles in peripheral blood lymphocytes using PAS staining is an inexpensive and rapid test to support a diagnosis of Pompe disease.[67]

To complete the diagnostic work-up after the demonstration of an acid α-glucosidase deficiency, DNA analysis is performed.[68] This information is also needed for facilitating genetic counseling of patients and their family. By DNA analysis the pathogenic sequence variations in GAA gen are detected, provided that each of them is located on another GAA allele.[68] The latter can be confirmed by establishing the parental segregation pattern. In case a diagnosis is made through DNA analysis, it is highly recommended to confirm the diagnosis by enzymatic testing.

(Co-incidental) findings of several tests may prompt suspicion of Pompe disease. Routine blood tests can be of supportive value. Patients with classic infantile Pompe disease, but also older children and adults - although not always-, have elevated serum levels of creatine-kinase (CK), transaminases (AST and ALT), and lactate dehydrogenase (LDH). Normal CK values are most often encountered in patients with advanced disease, and seldom in the early stages of the disease.[10 26 36 69 70]

Given that clinical symptoms are due to muscle weakness and that elevated serum CK levels occur in Pompe patients, muscle biopsy is frequently used in the diagnostic process. The muscle biopsy of a patient with Pompe disease usually shows a vacuolar myopathy (**Figure 4;** see also the paragraph about pathology). The vacuoles have increased glycogen contents, which can be visualized by PAS-staining, and are identified as
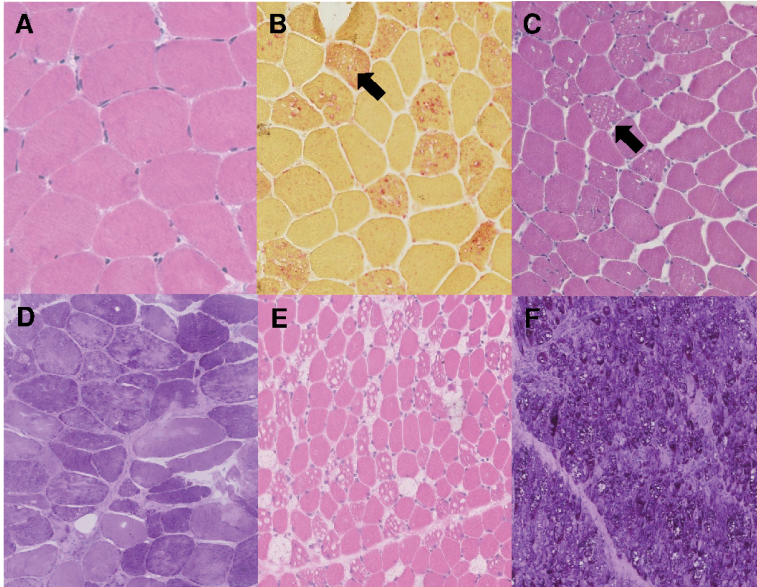
Figure 4 Muscle biopsies of a healthy control and patients with Pompe disease
A shows a Haematoxylin-Eosin (HE) stain of a cross section of a muscle biopsy from muscle tissue of a healthy individual. The muscle architecture is normal and there are no signs of myopathic changes. **B** is an acid phosphatase staining and **C** is a HE staining of a cross section of muscle biopsy of an adult patient with a confirmed diagnosis of Pompe disease. Multiple muscle fibers show small vacuoles (arrow), typical for a vacuolar myopathy. In Pompe disease these vacuoles are enlarged lysosomes, filled with glycogen, which show on the Periodic Acid-Schiff (PAS) staining (**D**) as a deep purple color. In **B-D** muscle cell morphology is essentially preserved, except for some variance in muscle fiber size, which indicates slight myopathic changes. **E** shows a HE staining of a severely affected classic-infantile patient showing the clear variation in muscle fiber size and vacuolar myopathy. **F** shows a PAS staining of a muscle biopsy from a severely affected classic-infantile patient, the deposition of glycogen has massively destructed the muscle morphology, and only very few fibers are well preserved. Muscle fibers contain multiply enlarged lysosomes.

lysosomes by the positive reaction for acid phosphatase. The acid phosphatase staining is the most sensitive stain for showing the vacuolar myopathy (**Figure 4B**), as in adult Pompe disease this stain can show pathologic changes, while the PAS-staining does not. It is important to know that a normal muscle biopsy does not rule out a diagnosis of Pompe disease, since approximately up to 30% of adult patients' muscle biopsies can be non-pathological.[33]

Hence, when the differential diagnosis on beforehand includes Pompe disease, the first step should be to apply for an enzyme assay, as this is a less invasive and quicker method for diagnostic confirmation.

PATHOGENESIS

Pompe disease is caused by pathogenic sequence variations in the gene coding for acid α-glucosidase, which is located on chromosome 17q25.2-q25.3 (Online Mendelian Inheritance in Man [OMIM] number 232300).[71] The pattern of inheritance is autosomal-recessive, this means that both GAA alleles need to carry a pathogenic mutation before the disease develops. Patients have inherited a pathogenic mutation from each parent. The mutations can be the same or different. In the latter case the patients are compound heterozygotes. Parents are true carriers in virtually all family situations, and carriers of Pompe disease are unaffected.[35] In very rare situations one of the two parents carries a second pathogenic mutation next to the one transmitted to the child and can be affected too. Thus far over 400 sequence variants in the GAA gene have been described (see www.pompecenter.nl), 70% of these are pathogenic. Most mutations are very rare, but some prevail within ethnic groups.

The sequence variants in the GAA gene vary by severity. Due to degenerate codons some nucleotide changes do not have any effect on the nature of the encoded amino acid. Missense mutations substitute one amino acid for another and have, in extremo, no effect at all or lead to total loss of protein function. Frame shift mutations, stop codons, and large deletions/insertion are mostly detrimental for protein function. Thus, the kind and combination of mutations in each of the two copies of the GAA genes determines how much of either catalytically active or inactive acid α-glucosidase is produced in the cells of a patient with Pompe disease.

The patients with classic infantile Pompe disease have virtually no acid α-glucosidase activity left, whereas patients with first symptoms in adulthood have in general residual acid α-glucosidase activity up to 25% of normal.

The most common and most severe pathogenic GAA sequence variants in the Dutch population are: c.2481+102_2646del (delexon18; p.Gly828_Asn882del), c.525del (delT525; p.Glu176fsX45), and c.925 G>A (p.Gly309Arg).[22] The most common mutation in patients with onset of symptoms in childhood or adulthood is: c.-32-13T>G (IVS1), a mutation located in intron 1 of the GAA gene reducing the fidelity of correct splicing to 10-20% of average normal, which reduces the acid α-glucosidase production to the same extent.[72-75]

Thus, there is a scale of residual acid α-glucosidase activities along the clinical spectrum of Pompe disease; very low in early onset disease and higher in later onset phenotypes. However, some patients with onset of symptoms in late adulthood were demonstrated to have very low acid α-glucosidase activity, which points to the rather strong effect that secondary factors can have on the clinical expression of Pompe disease. Phenotypic diversity has also been reported within families.[35 57 76]

Pathology

When there is virtually no acid α-glucosidase activity left, as in classic infantile Pompe disease, the lysosomal glycogen degradation is completely blocked. Lysosomal glycogen starts to accumulate rapidly in all tissues, although symptoms arise mainly from the accumulation in skeletal, cardiac and smooth muscle.[20][22] In children and adults with Pompe disease, the lysosomal glycogen accumulation has primarily pathogenic consequences in the muscle fibers of the proximal muscles. The reduced pulmonary function is mainly caused by weakness of the diaphragm.[38] Cerebral or thoracic aneurysms or dissections can occur due to accumulation of lysosomal glycogen in the smooth muscle cells of the vascular wall.[77-81]

The process by which lysosomal glycogen storage leads to loss of muscle contractile function is still a point of interest as knowing the precise mechanism may open the way to new treatment options.

Cytoplasmic glycogen stores in liver and muscle tissue and serves as quickly available energy resources, but lysosomal glycogen turnover does not seem to be of crucial importance for energy supply. Unlike patients with McArdle disease, patients with Pompe disease do not suffer from cramps or muscle pain during exercise and they have a normal glycogen metabolism during exercise.[82] Mechanical distortion of muscle contraction was demonstrated to be the more likely mechanism of muscle weakness in Pompe disease. In the early phase of the disease process, glycogen-filled lysosomes may settle in between the myofibrils and reduce contractile force.[83] When lysosomal glycogen storage continues, the number of lysosomes increases as well as their size. They may ultimately rupture thereby releasing lytic enzymes into the cytoplasm.[84] Along with lysosomal dysfunction, and probably as a result of it, the autophagic pathway is derailed.[85] This leads to deposition of non-contractile cellular debris disrupting muscle architecture and muscle contractibility.[49] As a consequence the muscle performance decreases and the muscle fibers become atrophic. Finally, the muscle fibers are replaced by connective and adipose tissue, and irreversible damage has occurred. **Figure 4B-D** show different stainings of a cross section of a muscle biopsy of an adult patient, showing the picture of a mild vacuolar myopathy, where multiple muscle fibers show small vacuoles. **Figure 4F** shows a Periodic Acid-Schiff (PAS) staining of a muscle biopsy from a severely affected classic-infantile patient. The deposition of glycogen has caused massive destruction of the muscle tissue, and only very few fibers are well preserved.

TREATMENT

The treatment of patients with Pompe disease can be divided into disease-specific and supportive treatment. Enzyme replacement therapy with alglucosidase alfa is currently the only registered disease-specific treatment, nevertheless the following treatment options are among the strategies currently being studied: alternative formulas of recombinant human α-glucosidase with improved efficacy; gene-modified autologous hematopoietic stem-cell therapy; and pharmacological chaperone therapies. These new treatment strategies will be addressed in the general discussion of this thesis.

Supportive treatment

Before the introduction of ERT, supportive care for the classical-infantile patients was mainly targeted at respiratory support, providing adequate nutrition and alleviating symptoms, and had a less multidisciplinary character than the supportive care for children and adults with the disease. With the advent of ERT, however, classic-infantile patients live longer and exhibit a new phenotype with residual morbidity also creating a need for multidisciplinary care.

The multidisciplinary team, often consists of a pediatrician (children) or internist (adults), (child)neurologist, pulmonologist, and a rehabilitation physician. Because the clinical manifestations and course of Pompe disease in children and adults are fairly variable, supportive management and rehabilitation should be individualized. The rehabilitation program is targeted at preserving and optimizing motor and respiratory function. Therefore the following services should be available: physical therapy, occupational therapy, speech therapy with dysphagia evaluation, nutritionist, orthotics, and psychological support.

For patients with symptomatic pulmonary dysfunction mechanical ventilatory support ought to be timely installed. As diaphragmatic weakness is a hallmark of the disease, patients often suffer from nocturnal hypoventilation and sleep-disordered breathing.[86] By installing nightly non-invasive ventilation, patients' general well-being will significantly improve and safeguard the patient from acute respiratory failure.[39][87]

Enzyme replacement therapy

In the 1960s unsuccessful attempts were made to treat patients with intravenous infusions of acid α-glucosidase preparations from human placenta and from Aspergillus niger. As we know now, the dosage was far too low and the preparations did not have the proper characteristics.[88-92] Since 1984 our research group at Erasmus MC has worked on the development of ERT for Pompe disease. By cloning the GAA gene and unraveling the biosynthesis, processing and lysosomal targeting of acid α-glucosidase, the production

of recombinant human α-glucosidase became feasible.[93-98] Two production lines were simultaneously explored: in genetically modified CHO cells (Chinese hamster ovary) and in the milk of transgenic rabbit. After proof of principle in animal models of Pompe disease, clinical application of ERT finally ushered at the turn of the twenty-first century. In 2006, approval from both the European and US regulatory authorities (EMA and FDA) was obtained for the use of alglucosidase alfa (Myozyme®) as long-term treatment for patients with a confirmed diagnosis of Pompe disease, and the treatment costs in the Netherlands were also reimbursed by the Dutch Health Insurance Companies (CVZ/CTG). In 2013, the Ministry of Health decided to prolong reimbursement based on the results of the randomized-controlled trial, of the open-label prospective study on the effects and safety of ERT in children and adults, and the study on survival in treated adults,[99-101] as well as the results of the farmaco-economic study.

Enzyme replacement therapy in classic infantile Pompe disease

Clinical trials of ERT with recombinant human α-glucosidase started in 1999.[102-109] Patients with classic infantile Pompe disease were treated first. They demonstrated improved survival, diminished cardiac hypertrophy, and gain in motor milestones. Several patients learned to roll over, stand and walk.[25 26] Today, there is over 15 year of experience with treating classic-infantile patients with ERT. Experiences collected over the entire period showed that about one third of treated patients learned to walk, but that about the same percentage of patients did not survive beyond the age of four years or became ventilator dependent. From these results we have learned that clinical outcome can be improved by an early start of treatment - when the patient is still in a relatively good condition - and higher dosing of alglucosidase alfa (40 mg/kg every week instead of 20 mg/kg every other week (eow)). Factors that adversely affect treatment outcome are a lack of Cross Reacting Immunological Material (CRIM), which is in essence any form of endogenous acid α-glucosidase, and a strong immunologic response to the administered recombinant enzyme.[104 109-113] Presently, measures are being taken to prevent or counteract these negative factors as much as possible.[114-117] With the implementation of an early treatment start, optimized dosing and immune tolerance protocols, the beneficial effects of ERT have substantially increased in patients with classic infantile Pompe disease as compared to the earlier days. However, variations in treatment responses are still observed and in the majority of patients some form of residual disease remains.[118-120]

Enzyme replacement therapy in children and adults with Pompe disease

The first pilot study in non-classic Pompe disease that started in 1999 at the Erasmus MC included two children and one adult patient. At start of treatment all three patients were severely affected: all were wheelchair bound and two of them used mechanical

ventilator support. They were initially treated with enzyme extracted from the milk of transgenic rabbits and switched in 2002 to enzyme derived from genetically modified CHO cells. Over 8 years of therapy, positive effects were observed on muscle strength and muscle function. The effects of ERT were minimal in the eldest patient who was almost paralytic at the start of treatment, but it was remarkable in the youngest, 11-year-old and least affected, patient. He learned to walk and has at present, after 15 years of ERT, virtually no symptoms. All three patients were less fatigued and experienced an improved quality of life.[121 122] The effects observed in classical infantile patients together with the positive results in the first observational studies performed in children and adults with Pompe disease,[121-126] led to the design of the first and only performed placebo-controlled clinical trial, named Late-Onset Treatment Study (LOTS).[101] This study evaluated the efficacy and safety of alglucosidase in 90 patients aged between 10 and 70 years of whom 60 received treatment and 30 placebo over an 18-month follow-up period. The study provided proof of efficacy, as treated patients showed improvement of walking distance and stabilization of pulmonary function and the results differed significantly from the placebo group. Results for the other outcome measures were also in favor of the treatment group, but statistical significance was only reached for improvement of maximal expiratory pressure (MEP). Patients in the treatment group and placebo group had similar frequencies of adverse events. Anaphylactic reactions occurred only in patients who received alglucosidase alfa (5%). All treated patients developed anti-alglucosidase alfa antibodies, but no consistent effect on treatment outcome or association with the occurrence of infusion-associated reactions was found.

The overall conclusion that can be drawn from the clinical studies summarized in **Table III** of the general discussion (**Chapter 5**) is that about 60-90% of the patients receiving ERT experience stabilization or improvement of skeletal muscle strength, motor function and pulmonary function, even though effects are modest. Considering the progressive nature of the disease clinical stabilization should be regarded as a positive outcome.[127] The latest important finding is that ERT prolongs the survival of affected adults.[100]

As in classic infantile Pompe disease, some children and adults respond far better to ERT than others. The reason why is a challenging subject for future research. By monitoring the long-term treatment effects and determining prognostic factors for treatment outcome, the goal is to optimize treatment efficacy and develop proper treatment guidelines, thereby further improving patients' perspectives.

SCOPE

When enzyme-replacement therapy was about to become available for patients with Pompe disease, our research activities were strongly focused on data collection from untreated patients to enable later comparison of the clinical course before and after treatment. In 2004, just before enzyme replacement therapy became available, the Dutch nationwide prospective observational study on the natural course and the effects of enzyme replacement therapy in children and adults with Pompe disease started at Erasmus MC. In addition, information on level of handicap, quality of life and fatigue is gathered through questionnaires. The latter activity is conducted via the IPA (International Pompe Association)/Erasmus MC Pompe survey, an ongoing international survey that already started in 2002. In this survey, patients from around the world participate and are yearly questioned.

Up to now, our center has coordinated the care for 160 Pompe patients, of whom 136 received enzyme replacement therapy. In the context of the nationwide prospective cohort study, all patients are followed on fixed regular intervals using a large set of assessments (**Appendix A**).

The results described in this thesis are predominantly based on patient-related data collected in the Erasmus MC natural course prospective cohort study, and on the subsequent study on the effects of ERT in adults with Pompe disease. **Chapter 2.2** and **Chapter 4.3** report on data collected in the context of the IPA/Erasmus MC Pompe survey.

Study aims

The aims of the studies described in this thesis were as follows:

1. To investigate and to describe the clinical features of adults with Pompe disease and follow how these features develop over time.
2. To study the effects of enzyme replacement therapy and to identify prognostic factors related to treatment response.
3. To investigate the impact of adverse events and antibody response to recombinant human acid α-glucosidase in adults affected by Pompe disease.
4. To study whether continued enzyme replacement therapy during pregnancy and lactation is safe for mother and child.
5. To study the impact of fatigue in neuromuscular diseases with a focus on Pompe disease and to evaluate the effect of enzyme replacement therapy on fatigue in adults with Pompe disease.

Outline of the thesis

Chapter 1 is an introductory chapter providing elementary information on Pompe disease. Besides the clinical features, the spectrum of the disease, enzymatic and molecular background, muscle pathology, and diagnostic tools, aspects of treatment with enzyme-replacement therapy are described. **Chapter 2.1** delineates the natural disease course in 94 adults with Pompe disease. **Chapter 2.2** deals more specifically with survival, and reports the survival data of a large international cohort of untreated patients. **Chapter 3** is dedicated to enzyme replacement therapy. The effects of enzyme replacement therapy and prognostic factors for treatment outcome are described in **part 3.1**; the role of antibody response and the occurrence of infusion-associated reactions are highlighted in **part 3.2** and **part 3.3**; while **part 3.4** is about enzyme-replacement therapy during pregnancy and lactation. **Chapter 4** explores the causes of fatigue and the burden of fatigue in neuromuscular disorders with special emphasis on Pompe disease. The effect of enzyme replacement therapy on fatigue in Pompe disease is described at the end.

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2.1

CHAPTER 2.1

Clinical features and predictors for disease natural progression in adults with Pompe disease: a nationwide prospective observational study

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ABSTRACT

Background Due partly to physicians' unawareness, many adults with Pompe disease are diagnosed with great delay. Besides, it is not well known which factors influence the rate of disease progression, and thus disease outcome. We delineated the specific clinical features of Pompe disease in adults, and mapped out the distribution and severity of muscle weakness, and the sequence of involvement of the individual muscle groups. Furthermore, we defined the natural disease course and identified prognostic factors for disease progression.

Methods We conducted a single-center, prospective, observational study. Muscle strength (manual muscle testing, and hand-held dynamometry), muscle function (quick motor function test), and pulmonary function (forced vital capacity in sitting and supine positions) were assessed every 3–6 months and analyzed using repeated-measures ANOVA.

Results Between October 2004 and August 2009, 94 patients aged between 25 and 75 years were included in the study. Although skeletal muscle weakness was typically distributed in a limb-girdle pattern, many patients had unfamiliar features such as ptosis (23%), bulbar weakness (28%), and scapular winging (33%). During follow-up (average 1.6 years, range 0.5–4.2 years), skeletal muscle strength deteriorated significantly (mean declines of -1.3% point/year for manual muscle testing and of -2.6% points/year for hand-held dynamometry; both $p<0.001$). Longer disease duration (>15 years) and pulmonary involvement (forced vital capacity in sitting position <80%) at study entry predicted faster decline. On average, forced vital capacity in supine position deteriorated by 1.3% points per year ($p=0.02$). Decline in pulmonary function was consistent across subgroups. Ten percent of patients declined unexpectedly fast.

Conclusions Recognizing patterns of common and less familiar characteristics in adults with Pompe disease facilitates timely diagnosis. Longer disease duration and reduced pulmonary function stand out as predictors of rapid disease progression, and aid in deciding whether to initiate enzyme replacement therapy, or when.

BACKGROUND

Pompe disease is a rare autosomal recessive metabolic disorder, whereby mutations in the GAA gene lead to partial or total absence of the lysosomal enzyme acid α-glucosidase. The disease presents as a spectrum of phenotypes, ranging from a rapidly fatal phenotype in infants[1] to slower progressive phenotypes in older children and adults.[2,3] Many adults with Pompe disease are diagnosed late in life, when they are already in an advanced stage of the disease. While this may be due to clinicians' unawareness, it may also be explained by similarities in clinical presentation with other 'limb-girdle' diseases such as the limb-girdle muscular dystrophies (LGMD), Becker muscular dystrophy, or metabolic myopathies.[4] Our first objective was to optimize future diagnosis in this patient group by classifying their specific clinical features, and by delineating the distribution and severity of muscle weakness and the sequential involvement of individual muscle groups during the course of the disease.

Since 2006, enzyme replacement therapy (ERT) with recombinant human acid α-glucosidase has been approved for the treatment of Pompe disease. In infants, treatment generally improves cardiorespiratory function and motor function, and prolongs survival.[5-8] In older children and adults ERT was shown to improve or stabilize skeletal muscle strength, muscle function and respiratory function. However, the magnitude of the therapeutic response varies between individual patients.[9-14] To fully assess the effects of enzyme therapy, and to decide whom to treat and when to start treatment, it is necessary to be optimally informed about the course of disease prior to treatment, and about factors influencing disease progression. We thus prospectively studied the natural disease course, and aimed to identify prognostic factors for faster disease progression and poor outcome in a large cohort of adult Pompe patients.

METHODS

Participants and study design

We performed a single-center, prospective, cohort study, in which participation was open to all adults diagnosed with Pompe disease who had not yet received treatment with enzyme replacement therapy. Their diagnosis was confirmed by acid α-glucosidase assay in leukocytes or fibroblasts and by mutation analysis. All patients were seen between October 2004 and August 2009 at Erasmus MC University Medical Center, the designated center of expertise for Pompe disease in the Netherlands. The interval between visits was three to six months. Patients were recruited either through neuromuscular centers in the Netherlands and Belgium, through the Dutch neuromuscular patient organiza-

tion, or were referred to our Center by their treating physicians. The research protocol was approved by the Central Committee on Research Involving Human Subjects in the Netherlands (CCMO). All patients provided written informed consent.

Seven patients participated in the placebo arm of the randomized, placebo controlled trial on the safety and efficacy of alglucosidase alfa in late-onset Pompe disease.[13] Data on these patients collected during this period are included in the present analyses. We have previously reported long-term retrospective data on muscle strength and pulmonary function in 16 patients with Pompe disease.[15] While 12 of these patients participated in the current study, the present analyses were based solely on new, prospectively obtained data.

Procedures

We gathered information on the following: 1) the nature of first symptoms and the age at which these had presented; 2) the age at which the diagnosis had been made; 3) duration of disease since onset of first symptoms; 4) the presence of specific clinical features such as scoliosis, bulbar involvement (defined as weakness of muscles involved in speech, chewing and swallowing), winging of the scapula (defined as a clearly visible protrusion of the scapula when the patient was in a resting position or was lifting the arms anteriorly or sidewards), muscle atrophy, or ptosis; 5) the use of a wheelchair or walking aids; 6) skeletal muscle strength; 7) the use of mechanical ventilatory support; 8) the number of hours of ventilatory support per day; 9) pulmonary function; 10) cardiac function; 11) acid α-glucosidase activity in leukocytes and fibroblasts; 12) serum creatine kinase (CK); and 13) type of GAA mutation.

Skeletal muscle strength and muscle function

By manual muscle testing using the Medical Research Council (MRC) grading scale[16] (range 0–5; all patients were examined by NvdB or JdV), we measured 25 different muscle groups throughout the body to define the distribution of skeletal muscle weakness and the severity of involvement of the separate muscle groups. We calculated a sumscore (range 0–130) for the muscle groups that were involved most: neck extensors, neck flexors, and bilateral shoulder adductors, shoulder abductors, shoulder exorotators, shoulder endorotators, elbow flexors, elbow extensors, hip extensors, hip flexors, hip abductors, hip adductors, knee flexors and knee extensors. This score was subsequently converted to a percentage of the maximum possible score. Although the abdominal muscles and trunk muscles were frequently involved, we did not include these muscle groups in the MRC sumscore since they were difficult to grade.

Hand-held dynamometry (HHD) (Cytec dynamometer, Groningen, the Netherlands) was used as a second measure of muscle strength to examine the following muscle

groups: neck extensors, neck flexors and bilateral shoulder abductors, elbow flexors, elbow extensors, hip flexors, hip abductors, knee flexors and knee extensors. The value (Newton) measured in each muscle group was expressed as a percentage of the median strength of healthy females and males[17], and then combined into a sumscore by averaging these for all 16 muscle groups, producing a score between 0 and 100 percent.

Muscle function was assessed using the Quick Motor Function Test (QMFT).[18] A total score (range 0–64) was obtained by adding the scores of all items. This was then expressed as a percentage of the maximum score.

Pulmonary function

Forced vital capacity (FVC) was measured using a Lilly type pneumograph (Viasys Healthcare, Würzburg, Germany) or the KoKo spirometry system (Ferraris Respiratory, Louisville, USA) with the patient in upright seated and supine positions, according to ATS/ERS standards.[19] Results were expressed as a percentage of predicted normal values.[20] A measured value below 80% of the predicted value was considered to be abnormally low. Seven male patients who were invasively ventilated were artificially allotted a FVC value of 10% – just below the least observed value – since their omission might have led to biased results. These seven patients were however excluded from the longitudinal analysis.

Statistical analysis

Baseline characteristics are summarized using descriptive statistics. Differences between males and females, and between patients with and without scapular winging, were assessed using χ^2 tests (wheelchair use, use of mechanical ventilation, and presence of scoliosis, bulbar muscle weakness, scapular winging or ptosis) or Mann-Whitney tests (strength of individual muscle groups, MRC sumscore, HHD sumscore, QMFT sumscore, and FVC measured in sitting and supine positions). We used the Spearman's rank correlation coefficient (ρ) to calculate the relationships between residual enzyme activity and rate of decline in muscle strength and pulmonary function, and between serum CK activity and age, muscle atrophy and disease duration. Longitudinal analysis of muscle strength and pulmonary function was performed using repeated measures ANOVA (random coefficient models). The annual changes are expressed in absolute percentage points (pp/y). For subgroup analyses, patients were divided into groups on the basis of gender (male, female); wheelchair use (yes, no); use of mechanical ventilation (yes, no); age at first study visit (<50 or ≥50 years; taking the median as the cut-off point); disease duration (<15 or ≥15 years; taking the median as the cut-off point); MRC/HHD sumscore at study entry (categorization in tertiles); and FVC in sitting position at study entry (<80 or ≥80 % predicted). Analyses were performed with SPSS for Windows (version 15, SPSS

Inc., Chicago, IL) or SAS (version 9.1, SAS Institute Inc., Cary, NC). A p-value of ≤0.05 was considered statistically significant.

RESULTS

Study population

In order of referral, we included 91 adult Pompe patients from the Netherlands – representing virtually all known patients in the Netherlands – and three patients from Belgium. On average, there was a seven-year delay between the first noted symptoms of Pompe disease and the actual diagnosis. The characteristics of the study population are summarized in **Table I**.

Table I Characteristics of the study population (n=94)[a]

General characteristics	
Gender (males)	48 (51%)
Age at first study visit (years)	51.1 (38.3–60.6)
Age at onset of symptoms (years)	32.0 (25.5–40.0)
− < 18 years	10 (11%)
− ≥ 18 years	84 (89%)
Age at diagnosis (years)	40.2 (32.7–50.2)
Disease duration since onset of first symptoms at first study visit (years)	15.3 (7.7–24.7)
Time since diagnosis at first study visit (years)	9.2 (0.6–16.0)
− 0 to 5 years	41 (44%)
− 5 to 10 years	15 (16%)
− 10 to 15 years	10 (11%)
− > 15 years	28 (30%)
Use of walking aids	14 (15%)
Wheelchair use	30 (32%)
Age at start of wheelchair use (years)	49.0 (43-56)
Use of mechanical ventilation [b]	27 (29%)
Age at start of mechanical ventilation (years)	48 (38.5–57.5)
First symptoms noted [c]	
Skeletal muscle weakness	93 (99%)
− Difficulty running	30 (32%)
− Difficulty performing sports	22 (23%)
− Difficulty climbing stairs	24 (26%)
− Difficulty walking	15 (16%)
− Difficulty rising from an armchair	11 (12%)
− Difficulty rising from a lying position	9 (10%)

Table I *Continued*

First symptoms noted [c]	
Fatigue	17 (18%)
Muscle soreness / cramps	16 (17%)
Respiratory failure	1 (1%)
Clinical features	
Ptosis	22 (23%)
Bulbar muscle weakness[d]	26 (28%)
Scapular winging[d]	31 (33%)
Scoliosis	22 (23%)
Increased lumbar lordosis	62 (66%)
Prominent muscle atrophy	53 (56%)
– Shoulder girdle / upper arms	25 (27%)
– Trunk muscles	27 (29%)
– Pelvic girdle / Upper leg (Figure 1A)	40 (43%)
Laboratory parameters	
CK (U/l)	
– Males	449 (279–1040)
– Females	493 (237–715)
α-glucosidase activity in leukocytes (nmol glucose/h/mg protein) [e]	1.2 (0.4–2.2)
α-glucosidase activity in fibroblasts (nmol 4-MU/h/mg protein) [f]	13.0 (11.0–15.0)
Genotype	
– c.-32-13T>G / very severe or potentially less severe pathogenic mutation	92 (98%)
– c.671G>A / c.525del	1 (1%)
– unknown	1(1%)

4-MU=4-methylumbelliferyl-α-D-glucopyranoside
[a] Data are number (%) or median (IQR). [b] More men then women used mechanical ventilation (p=0.009).
[c] Two or more complaints were counted if these occurred within the same year. [d] More men than women had scapular winging and bulbar muscle weakness (p=0.001 and p=0.05). [e] control range 48 to 215 nmol glucose/h/mg protein. [f] control range 45 to 180 nmol 4-MU/h/mg protein.

Baseline measurements

Characteristic clinical features

A substantial number of patients had less familiar features of Pompe disease, such as bulbar muscle weakness (28%), prominent scapular winging (33%, **Figure 1B**), or ptosis – not accompanied by external eye-movement disturbances (23%; **Figure 1C**). Seventy-one percent of patients with scapular winging had bulbar muscle weakness, against 37% without scapular winging (p=0.001).
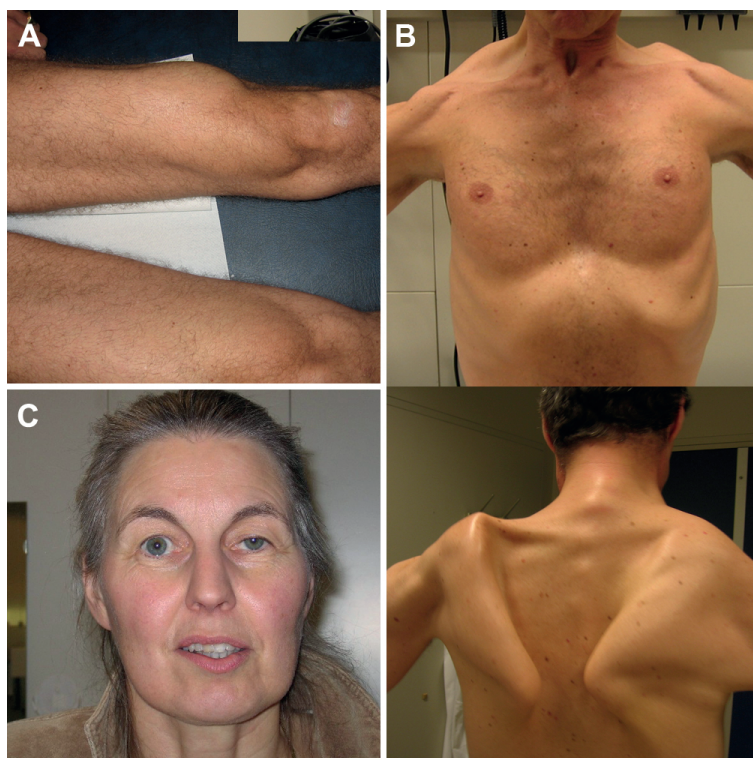
Figure 1 Clinical features in Pompe disease
Atrophy of the quadriceps muscle (**A**), scapular winging (**B**), and ptosis (**C**) as notable clinical features in adults with Pompe disease. Photographs are printed with permission of the patients.

Clinical distribution and severity of muscle weakness, and sequence of muscle involvement during the disease course

Shoulder abductors, abdominal muscles, paraspinal muscles, hip flexors, hip extensors, hip adductors, and hip abductors were affected in more than 80% of all patients (**Figure 2A**). The strength of the quadriceps muscle was reduced in only 55% of patients. The muscles of the hands and feet were relatively spared, being affected in under 10% of patients. Abdominal muscles, paraspinal muscles – with exception of the neck extensors and neck flexors –, hip flexors, hip extensors, hip adductors and hip abductors were the most severely affected muscles (**Figure 2B**). The pattern of muscle weakness was symmetrical in 95% of patients, and the distribution of weakness did not differ between males and females. Relative to patients without scapular winging, patients with scapular winging had more severe involvement of the shoulder girdle musculature (trapezius muscle, deltoid muscle, pectoral muscle, shoulder exorotators, and shoulder endorotators; all p<0.02). The 'limb-girdle' and trunk muscles were affected early in the course of
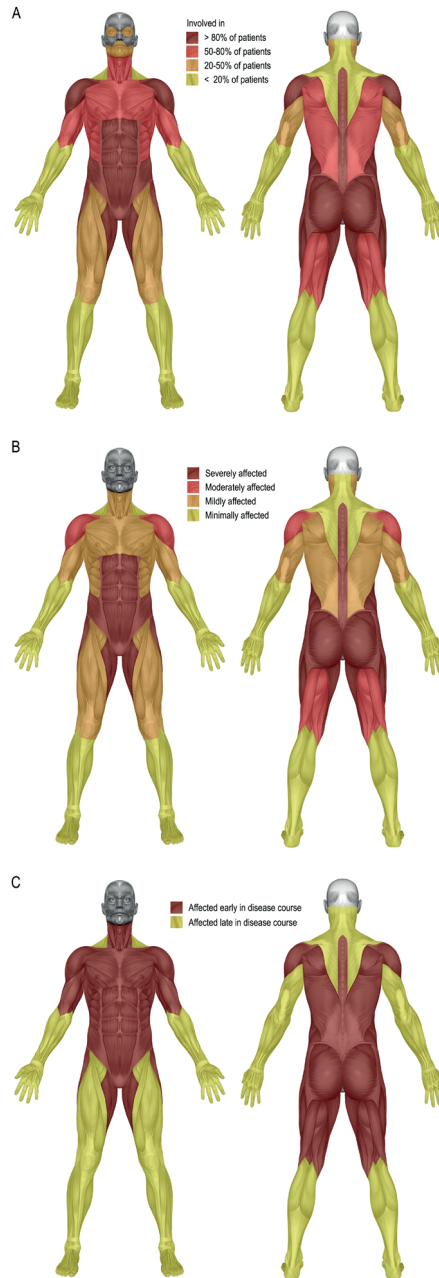
Figure 2 Muscle weakness in adults with Pompe disease

Distribution of skeletal muscle weakness (**A**), severity of muscle weakness of the individual muscle groups (**B**), and involvement of the individual muscles over time (**C**) in 94 adults with Pompe disease.

the disease, while the distal muscle groups – if they were involved at all – were affected late in the course of the disease (**Figure 2B**).

Cardiac evaluation

In a subset of 51 patients cardiac evaluation was performed, comprising electrocardiography, Holter monitoring, two-dimensional echocardiography, low-dose dobutamine stress echocardiography, and tissue Doppler imaging. One patient had a mild hypertrophic cardiomyopathy, while four other patients had minor cardiac abnormalities that could be attributed to advanced age, hypertension or preexisting cardiac pathology unrelated to Pompe disease.[21,22]

Laboratory parameters

Most patients had moderately increased serum CK; in two patients it was more than 10 times the upper limit of normal, while 10 had a normal serum CK. Serum CK activity was moderately inversely associated with age (ρ -0.71, $p<0.001$), disease duration (ρ -0.45, $p<0.001$), and the presence of muscle atrophy (ρ -0.53, $p<0.001$).

Prospective follow-up

General aspects

Prospective follow-up data for a period longer than six months were available for 66 patients (median 1.6 years, range 0.5–4.2 years), 52 of whom were followed for longer than one year, and 22 of whom for longer than two years. Within the follow-up period, one patient became wheelchair bound, mechanical ventilation was initiated in four patients, and eight patients needed to increase their number of hours of ventilation per day. One severely affected patient died due to pneumonia complicated by respiratory failure.

Disease progression and predictors for disease outcome

Muscle strength and muscle function

At baseline, the MRC sumscore ranged from 39.2% to 100% (median 84.7%, one patient had the maximum possible score). During follow-up, the MRC sumscore deteriorated by 1.3% points per year on average ($p<0.001$; **Figure 3A**). Baseline values for hand-held dynamometry ranged from 31.6% to 100% (median 77.0%, three patients had the maximum score). Within the observed follow-up period, HHD sumscore deteriorated by 2.6% points per year ($p<0.001$; **Figure 3B**). With regard to the individual muscle groups, strength declined significantly in the elbow flexors, hip abductors, knee extensors and knee flexors, ranging from -6.9 pp/y in elbow flexors to -2.5 pp/y in knee extensors
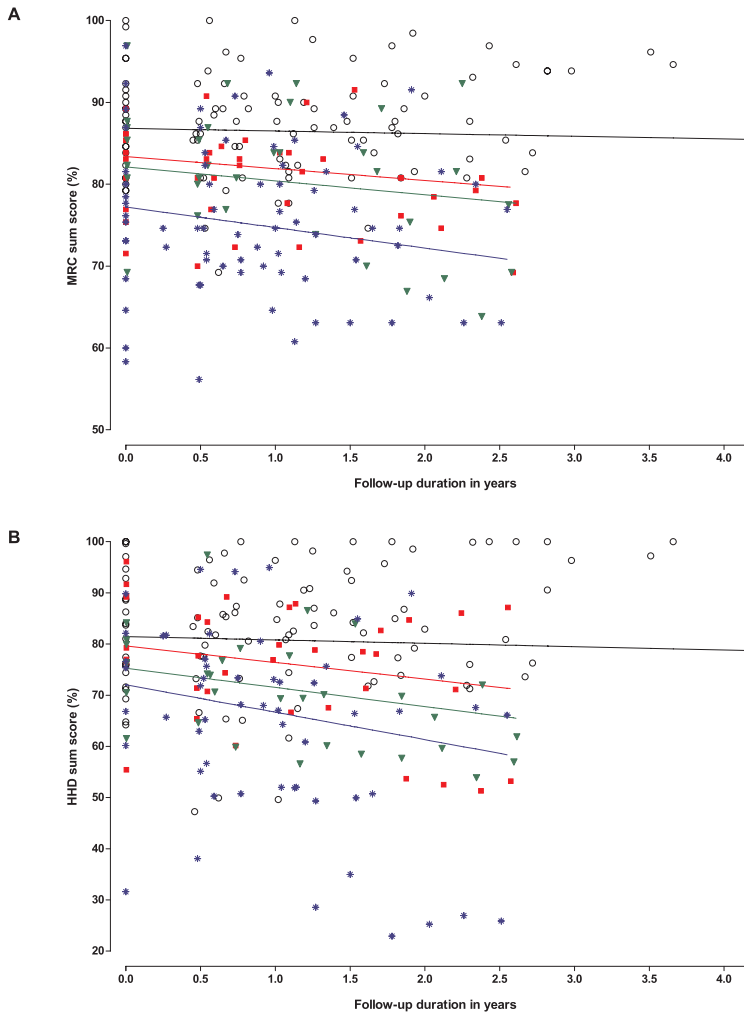
Figure 3 Longitudinal changes in muscle strength
Rate of disease progression measured by manual muscle testing (MRC sumscore) (A) and hand-held
dynamometry (HHD sumscore) (B) related to follow-up duration measured from time of inclusion in
the study for 66 adults with Pompe disease. The figure shows the measured values and regression lines
at group level for the following subgroups: 1) Patients with normal pulmonary function (FVC ≥80%
predicted) and disease duration <15 years (circles, black line); 2) patients with normal pulmonary function
(FVC ≥80% predicted) and disease duration ≥15 years (red squares, red line); 3) patients with abnormal
pulmonary function (FVC <80% predicted) and disease duration <15 years (green triangles, green line);
and 4) patients with abnormal pulmonary function (FVC <80% predicted) and disease duration ≥15 years
(blue asterisks, blue line).
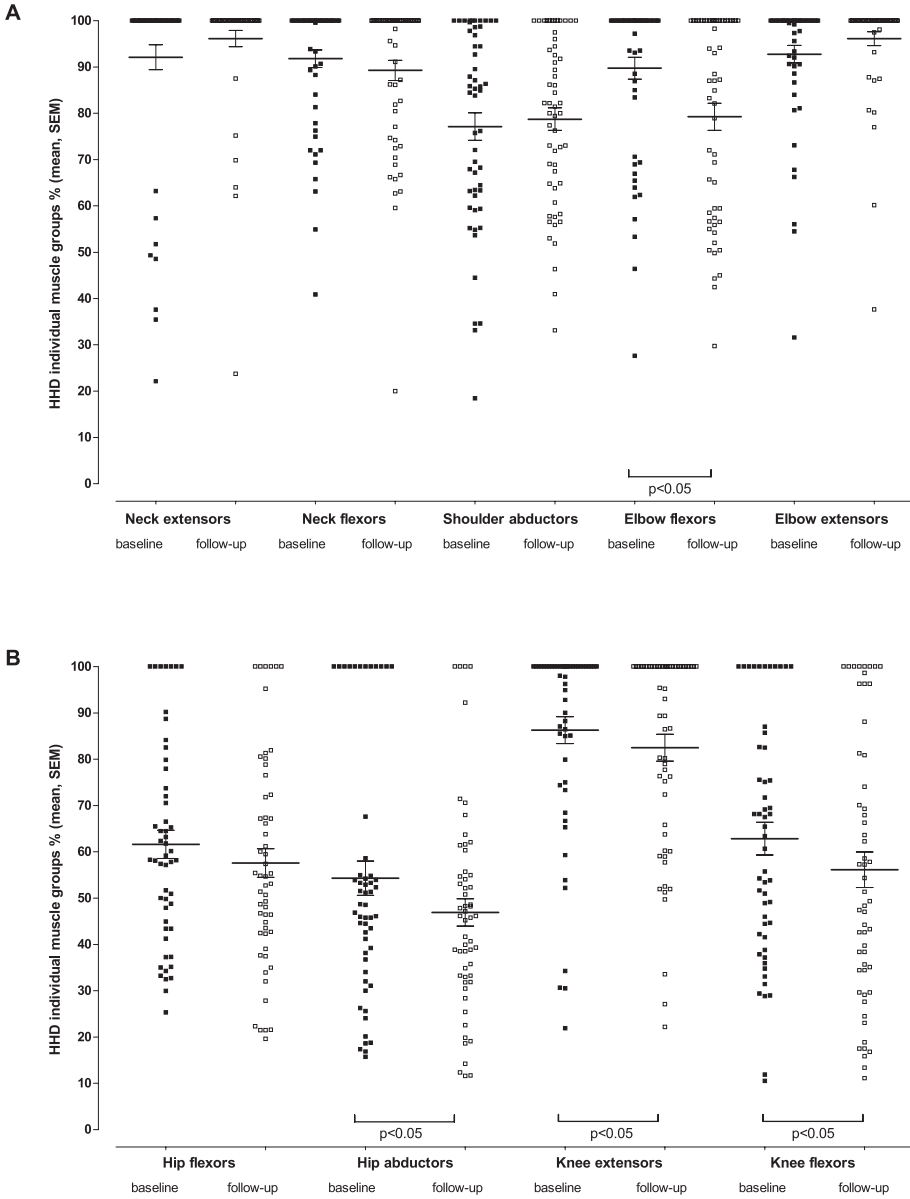
Figure 4 Muscle strength in individual muscle groups measured by hand-held dynamometry
Values for individual patients are shown at baseline (black squares) and during follow-up (last measured value) (open squares). Mean and standard error of the mean are given for each muscle group.

(**Figure 4**). Subgroup analysis (**Table II**) revealed that the decline was faster in patients with a reduced pulmonary function at baseline (FVC <80%) than in those with normal pulmonary function (-2.2 pp/y against -0.6 pp/y for MRC sumscore (p=0.01), and -4.5 pp/y against -1.4 pp/y for HHD sumscore (p<0.01)), and in patients who had had the disease for longer than 15 years compared to those who had been ill less long (-2.1 pp/y against -0.7pp/y for MRC sumscore (p=0.04), and -4.2 pp/y against -2.0 pp/y for HHD sumscore (p<0.01)). Baseline QMFT scores ranged from 16.7% to 100% (median 63.7%). The changes that were found in muscle strength were however not reflected in changes in the QMFT (annual change 0.05% points, p=0.9). In no subgroups was a significant decline observed.

Pulmonary function
At baseline, FVC measured in sitting position was reduced (<80% of the predicted value) in 56 patients (60%). The reduction in FVC was more prominent in males than in females (mean FVC 57.5 % predicted against 80.3 % predicted, p<0.001). Patients with scapular winging had significantly lower FVC than those without scapular winging (mean FVC 50.7% predicted against 84.5% predicted, p<0.001). In supine position, 76 patients (80%) had a reduced FVC. Changing from a sitting to a supine position, FVC fell in 21 patients by over 25%, indicating possible diaphragmatic weakness. Neither was testing in the supine position attempted in 12 patients whose pulmonary function was already severely restricted in sitting position. We have recently reported more detailed data on pulmonary function in some of the study cohort.[23] The mean yearly change in FVC measured in supine position was 1.3% points (p=0.02), and for FVC in upright position -1.0% points (p=0.06) (**Figure 5**). The rate at which pulmonary function declined was consistent across subgroups.

There was no significant association between the change in muscle strength or pulmonary function and residual enzyme activity (Spearman's rho for MRC sumscore -0.21, p=0.14; for HHD sumscore -0.51, p=0.74; for FVC in upright position 0.07, p=0.65; and for FVC in supine position -0.32, p=0.84).

Table II Disease progression in 66 adults with Pompe disease: annual changes in muscle strength and pulmonary function across various subgroups

Total group	MRC sumscore			HHD sumscore			QMFT score		
	n	Annual change (%/year)	95% CI	n	Annual change (%/year)	95% CI	n	Annual change (%/year)	95% CI
	65	-1.30	-1.95 to -0.66	55	-2.6	-3.72 to -1.45	62	0.05	-0.76 to 0.86
Subgroups	n	Annual change (%/year)		n	Annual change (%/year)		n	Annual change (%/year)	
Gender									
Female	36	-1.41		31	-2.97		37	-0.22	
Male	29	-1.08		24	-1.99		25	0.57	
Age at study entry[a]									
< 50 years	34	-1.39		31	-2.06		33	-0.17	
≥ 50 years	31	-1.21		24	-3.29		29	0.30	
Disease duration at study entry[a]									
< 15 years	34	**-0.70[c]**		31	**-1.95[e]**		34	-0.07	
≥ 15 years	31	**-2.09[c]**		24	**-4.18[e]**		28	0.17	
FVC (sitting) at study entry									
≥ 80% predicted	33	**-0.62[d]**		32	**-1.43[f]**		33	-0.21	
< 80% predicted	32	**-2.21[d]**		23	**-4.52[f]**		29	0.31	
Ventilation at study entry									
No	49	-1.12		46	-2.50		50	0.24	
Yes	16	-1.83		9	-3.55		12	-0.70	
Wheelchair use at study entry									
No	47	-0.94		44	-2.28		47	-0.39	
Yes	18	-2.36		11	-3.92		15	1.44	
MRC/HHD sum score at study entry[b]									
< 33rd percentile	18	-1.27		10	-2.06		20	0.49	
33rd to 66th percentile	23	-1.31		22	-4.34		23	-0.06	
≥ 66th percentile	24	-0.98		23	-1.56		19	-0.41	

Table II *Continued*

	FVC sitting position			FVC supine position		
Total group	n	Annual change (%/year)	95% CI	n	Annual change (%/year)	95% CI
	59	-1.04	-2.14 to 0.06	54	-1.30	-2.42 to -0.19
Subgroups	n	Annual change (%/year)		n	Annual change (%/year)	
Gender						
Female	36	-1.38		33	-2.27	
Male	23	-0.45		21	0.35	
Age at study entry[a]						
< 50 years	33	-1.34		31	-2.22	
≥ 50 years	26	-0.69		23	-0.08	
Disease duration at study entry[a]						
< 15 years	35	-1.36		33	-1.37	
≥ 15 years	24	-0.60		21	-1.16	
FVC (sitting) at study entry						
≥ 80% predicted	33	-1.09		31	-1.59	
< 80% predicted	26	-0.89		23	-0.92	
Ventilation at study entry						
No	50	-1.30		47	-1.50	
Yes	9	1.07		7	0.32	
Wheelchair use at study entry						
No	47	-0.98		43	-0.99	
Yes	12	-1.22		11	-2.57	
MRC/HHD sum score at study entry[b]						
< 33rd percentile	12	-0.30		9	-1.59	
33rd to 66th percentile	23	-2.40		21	-2.01	
≥ 66th percentile	24	-0.34		24	-0.63	

MRC=Medical Research Council, HHD=Hand-Held Dynamometry, QMFT=Quick Motor Function Test, FVC= Forced Vital Capacity, CI=confidence interval. [a] the median was taken as the cut-off point. [b] categorization in tertiles. n represents the number of patients for whom data were available for each specific measurement. For muscle strength, no sumscore was calculated if measurements for three or more individual muscle groups were missing (e.g. due to severity of muscle weakness or injury). For seven invasively ventilated patients no reliable measurements of FVC could be performed. Neither could testing in the supine position be endured by 5 patients whose pulmonary function was already severely restricted in sitting position. Data shown are mean annual changes (percentage points per year) as calculated by repeated measures ANOVA. For MRC sumscore and HHD sumscore there were significant differences between groups based on disease duration and FVC at study entry: c) $p=0.04$, d) $p=0.01$, e) $p<0.01$, and f) $p<0.01$.

A

B
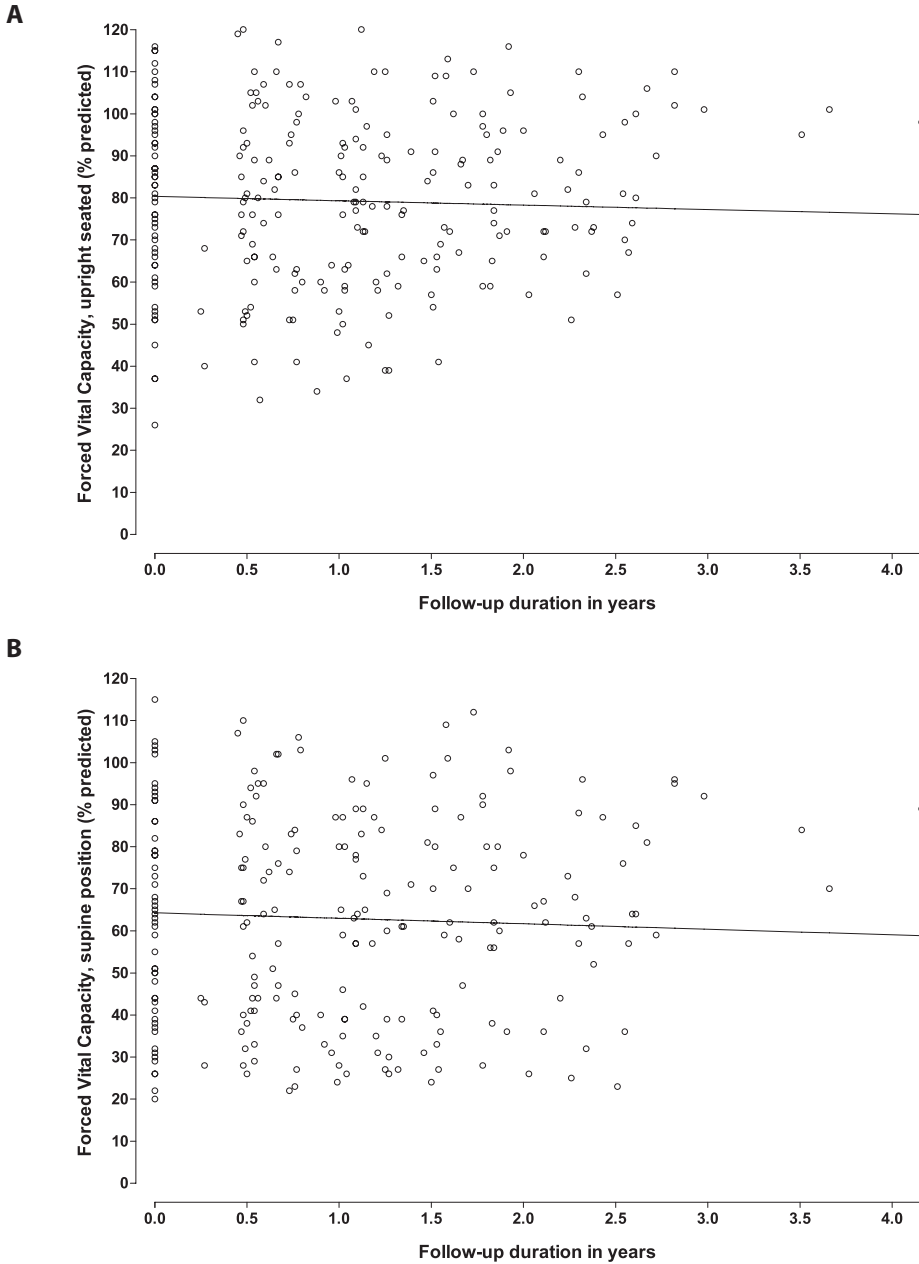
Figure 5 Longitudinal changes in pulmonary function
Decline in pulmonary function in the upright seated (**A**) and supine (**B**) positions related to follow-up duration. Circles represent the measured values, the line represents the mean regression line at a group level.

Disease course variability

In 59 patients progress of muscle weakness and pulmonary dysfunction could be compared. In nine patients (15%; 4 males, 5 females), pulmonary function and muscle strength did not decline during the prospective follow-up period. Relative to the total group, they had a shorter disease duration from onset of symptoms (7.3 years against 15.5 years, p=0.03). At baseline, the following had all been higher, though not significantly: FVC in sitting position (88.1% against 78.6%); FVC in supine position (80.4% against 61.1%); MRC sumscore (87.2% against 83.4%); and HHD sumscore (86.2% against 76.3%). In 28 patients (47%), pulmonary function and skeletal muscle strength declined at a similar rate. In 22 patients (37%) the course of pulmonary function and muscle weakness followed different courses, one deteriorating while the other remained stable, or one deteriorating faster than the other. Of the 50 patients who deteriorated during the follow-up period, eight (2 males, 6 females) had a relatively fast decline – more than 5 percentage points per year – in muscle strength, while a rapid decline in pulmonary function was seen in five patients (1 male, 4 females). There were no significant differences between these groups with regard to sex, age, age at onset of symptoms, duration of disease, length of prospective follow-up, level of residual enzyme activity, and the severity of pulmonary or skeletal muscle involvement at the start of the study.

DISCUSSION

Characteristic clinical features and pattern of muscle weakness

Our study in 94 adults with Pompe disease – which included virtually all known adult patients in the Netherlands – shows that, generally speaking, muscle weakness typically fits a pattern of limb-girdle myopathy. Interestingly, our findings about the distribution of muscle weakness, based on clinical examinations, match those of CT and MRI studies.[24-26] We found weakness of the quadriceps muscle in 55% of patients only. This may have been due to our method of measuring muscle strength: whereas the advantage of the MRC score and HHD lie in the fact that they are easy for the clinician to use, quantitative muscle testing using QMT[13,27] may be more reliable in patients with only minor loss of strength.

Besides limb-girdle weakness, many patients had less familiar features, which are sometimes symptomatic of other neuromuscular diseases. Though ptosis has been reported in the literature in no more than seven adult patients to date[28-31], it was found in almost one quarter of our patients. Notably, while one might expect myogenic ptosis to be bilateral, it was unilateral in two-thirds of our patients.

Despite the fact that bulbar muscle weakness is reported only occasionally in adult Pompe patients[28,32], twenty-eight percent of the patients in our cohort had distinct bulbar muscle weakness. These patients are at risk of pulmonary complications.

One third of patients had prominent scapular winging, which is in line with several recent reports.[32,33] Many of these patients also had bulbar muscle involvement and severer weakness of the shoulder-girdle muscles than patients without scapular winging; these features are reminiscent of FSHD. If patients with such a "pseudo-FSHD" phenotype are overlooked, diagnosis may be delayed. However, the asymmetrical distribution of muscle weakness, the relatively common involvement of the distal muscles of the lower limbs, and the absence of severe or relatively severe pulmonary involvement in FSHD will generally allow the two diseases to be diagnosed correctly.[34]

Serum CK activity was normal in 10 patients, confirming that a normal serum CK does not rule out Pompe disease.[35-37] In early stages of the disease, however, normal serum CK is quite uncommon. Although we did not systematically perform electromyography or muscle biopsies, it is well known that these may reveal no or only non-specific abnormalities in a considerable number of patients.[38,39] Therefore, measurement of acid α-glucosidase activity and mutation analysis remain essential to confirm the diagnosis.

Natural disease course

Although the average follow-up duration of 19 months seems rather short for a chronic disorder such as Pompe disease, it is the longest prospective follow-up ever carried out in "late-onset" Pompe disease. Moreover, as many patients are now treated with enzyme replacement therapy, prospective data on the natural course of the disease over a longer period of time are now impossible to obtain.

We found a significant worsening of muscle strength, reflected by declines in MRC sumscore (mean decline -1.3 pp/y) and HHD sumscore (mean decline -2.6 pp/y). Although we found significant changes in muscle strength and some patients became wheelchair or ventilator dependent during follow-up, the QMFT did not indicate a deterioration in limb-girdle muscle function. There may be two main reasons for this. First, the decline in muscle strength may have been too small to cause changes in functional daily activities within the observed time-span. Second, although the QMFT showed a good discriminative ability and good responsiveness to change after start of enzyme replacement therapy, it may not be sensitive enough to reflect minor changes in strength in functional changes during the natural course.[18]

Mechanical ventilation became necessary in four of the 67 hitherto un-ventilated patients, eight patients needed to increase their number of hours of ventilation, and one patient died due to respiratory complications. Despite this indication that pulmonary function clearly worsened during the time-span of the study, our findings regarding the

yearly decline seem somewhat lower than those of other studies.[13,27] This may have been due to our method of patient selection: in the present study participation was open to all adult patients with a confirmed diagnosis of Pompe disease, including those with very limited pulmonary function to those who were invasively ventilated 24 hours a day. Although this reflects the spectrum of disease encountered in daily practice, one would expect further deterioration of pulmonary function in these patients to be minimal, thereby partly obscuring decline in the total group. Secondly, when enzyme replacement therapy became commercially available, we decided first to treat the most severely affected patients and patients with a rapid decline in pulmonary function and muscle strength, while patients with a slow disease course were started on ERT at a later stage. As a result, the length of prospective follow-up for the most severely affected patients was somewhat shorter (1.1 years on average) than for the least affected patients (1.7 years on average, the longest follow-up being 4.2 years). Another factor that could have influenced the estimated rate of decline is the fact that data on seven patients who participated in the placebo arm of the randomized controlled trial on the safety and efficacy of alglucosidase alfa in late-onset Pompe disease were included in the present analyses. However, a second analysis, excluding these patients to overcome a possible placebo effect, led to similar estimates of the rate at which muscle weakness and pulmonary dysfunction progress (not shown).

It should be noted that all patients except one had the c.-32-13T>G (IVS1-13T>G) mutation in combination with a null allele. Although this is the most common genotype in adult Pompe patients – present in 68-93% of patients[27,40,41] – the estimated rate of disease progression may not apply to patients with different genotypes.

Predictors for disease outcome and disease course variability

Longer disease duration (>15 years) and pulmonary dysfunction (FVC <80%) were shown to be associated with a faster decline in muscle strength. Our results thus support those of the only other prospective study in adult Pompe patients, which found baseline status and duration of illness to be the most important predictors of disease severity and disease progress.[27]

A subset of patients with shorter average disease duration and better baseline status did not deteriorate during follow-up, indicating that there might be a stable phase of several years before a gradual decline inevitably occurs. This raises an interesting dilemma regarding the best time to start ERT.[42] On the one hand, in patients who are only mildly affected and whose condition is stable, one might advocate that this – costly – lifelong treatment be postponed. On the other hand, studies measuring the effect of ERT show a trend toward better outcome in patients who were in a relatively good condition at the start of treatment.[11,13,14] On the basis of our results, we suggest to start ERT in all patients

with pulmonary dysfunction and in patients with progressive muscle weakness, whereas in patients with minimal weakness, or in patients with solely elevated laboratory parameters treatment may be postponed, provided that they are monitored regularly. At our center, all patients undergo evaluation of muscle strength, pulmonary function, cardiac function, and hearing at referral, and evaluation of progression of muscle weakness and pulmonary dysfunction every three months thereafter.

CONCLUSIONS

In summary, since the approval of enzyme replacement therapy in Pompe disease, early recognition of affected individuals has gained importance. The typical limb-girdle type muscle weakness – including prominent involvement of the trunk musculature – combined with early and disproportionate pulmonary involvement relative to the degree of skeletal muscle weakness should raise the suspicion of Pompe disease. Although these are the most salient characteristics, less familiar features such as ptosis, scapular winging and bulbar weakness are far more common than generally thought. If these are recognized properly, timely diagnosis will be facilitated. Longer disease duration and reduced pulmonary function stand out as the most important factors for a rapid decline in muscle strength, which may aid in deciding whom to treat and when.

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Chapter
2.1

2.2

CHAPTER 2.2

Survival and associated factors in 268 adults with Pompe disease prior to treatment with enzyme replacement therapy

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ABSTRACT

Background Pompe disease is a rare lysosomal storage disorder characterized by muscle weakness and wasting. The majority of adult patients have slowly progressive disease, which gradually impairs mobility and respiratory function and may lead to wheelchair and ventilator dependency. It is as yet unknown to what extent the disease reduces the life span of these patients. Our objective was to determine the survival of adults with Pompe disease not receiving ERT and to identify prognostic factors associated with survival.

Methods Data of 268 patients were collected in a prospective international observational study conducted between 2002 and 2009. Survival analyses from time of diagnosis and from time of study entry were performed using Kaplan-Meier curves and Cox-proportional-hazards regression.

Results Median age at study entry was 48 years (range 19-79 years). Median survival after diagnosis was 27 years, while median age at diagnosis was 38 years. During follow-up, twenty-three patients died prior to ERT, with a median age at death of 55 (range 23-77 years). Use of wheelchair and/or respiratory support and patients' score on the Rotterdam Handicap Scale (RHS) were identified as prognostic factors for survival. Five-year survival for patients without a wheelchair or respiratory support was 95% compared to 74% in patients who were wheelchair-bound and used respiratory support. In a Dutch subgroup of 99 patients, we compared the observed number of deaths to the expected number of deaths in the age- and sex-matched general population. During a median follow-up of 2.3 years, the number of deaths among the Dutch Pompe patients was higher than the expected number of deaths in the general population.

Conclusion Our study shows for the first time that untreated adults with Pompe disease have a higher mortality than the general population and that their levels of disability and handicap/participation are the most important factors associated with mortality. These results may be of relevance when addressing the effect of ERT or other potential treatment options on survival.

BACKGROUND

Pompe disease, synonymously 'acid maltase deficiency' or 'glycogen storage disease type II', is a metabolic myopathy caused by deficiency of the enzyme acid α-glucosidase and resulting in intralysosomal accumulation of glycogen. This autosomal recessive disorder is mainly characterized by progressive loss of muscle strength and respiratory function due to destruction of muscle tissue.[1, 2] Because of its low frequency of approximately 1 in 40,000 births and the broad ethnic spreading,[3-5] Pompe disease is a true orphan disease with the associated problem of collecting data in sufficiently large groups. Clinical heterogeneity is an additional complicating factor.[5, 6] Classic infantile Pompe disease, the most severe form, presents in the first months of life with generalized muscle weakness and cardiac hypertrophy. Without treatment these infants die before age one. Later-onset forms of Pompe disease comprise childhood, juvenile, and adult cases. The majority of these patients present with symptoms in adulthood with limb- girdle weakness and respiratory problems.[5]

For a long time, supportive care such as respiratory support was the only way of managing Pompe disease, but in the course of 2006 enzyme replacement therapy (ERT) with recombinant human α-glucosidase became available. Clinical trials showed that ERT can ameliorate motor outcome, improve cardiomyopathy and prolong survival in classic infantile Pompe disease.[7-11] In children and adults treatment with ERT has been shown to stabilize respiratory function and to improve muscle function.[12-16]

In contrast to classic infantile Pompe disease, in which survival is a key outcome measure to describe the natural course of the disease *and* to evaluate the effects of treatment, information on mortality in adults with Pompe disease has been lacking. The present study was performed to fill the gap of knowledge on the impact of Pompe disease on survival in untreated adult patients, using data collected prospectively in an international patient survey prior to the introduction of ERT. The objective was to determine natural course survival in adult patients with Pompe disease, to compare this to the general population and to assess differences in survival between subgroups of patients.

METHODS

Data
Data were collected between May 2002 and December 2009 as part of an ongoing study on the natural course of Pompe disease ('Pompe Survey'),[6, 17] e.g. in which patients

complete a number of self-report questionnaires each year, gathering information on medical history, current disease status, use of care and quality of life.

Patients were recruited through patient organizations affiliated with the International Pompe Association (IPA) in Australia, Canada, Germany, the Netherlands, the United Kingdom and the United States. Inclusion criteria for the Pompe Survey were a diagnosis of Pompe disease and an age above 2 years. The present analyses only include patients of 18 years and older with late-onset Pompe disease.

For the Dutch patients, more information was available as Erasmus MC was designated as the single referral center for treatment and longitudinal follow-up of Pompe patients in the Netherlands.

All research protocols were approved by the medical ethics committee of Erasmus MC and/or the Central Committee on Research Involving Human Subjects. Written informed consent was obtained from all patients.

Explanation of variables

For the international participants in the Pompe Survey, the date of the last completed questionnaire before December 2009 was considered as the date of last follow-up. For the Dutch subgroup, the date of last follow-up was the last visit at our hospital in 2009 or the date of the last completed questionnaire, whichever came last.

When the date of death for the deceased was not exactly known it was estimated to be halfway between the date of the last completed questionnaire and the date at which the next questionnaire should have been completed.

The date of diagnosis was estimated as precisely as possible according to the information provided in the questionnaires.

To assess the level of participation (defined as a person's involvement in life situations; previously called 'Handicap'),[18, 19] the Rotterdam Handicap Scale (RHS) was used. The RHS consists of 9 questions on the topics of mobility, kitchen tasks, domestic tasks, leisure activities, travelling and work or study. The scores per item range from 1 ('unable to fulfil the task or activity') to 4 ('complete fulfilment of the task or activity'). If an item is not applicable to a patient, a score of 0 is given. The total score is calculated as the sum of the scores per item * 9/ (9-number of non-applicable or missing items).[17, 20] The RHS score thus ranges from 9 to 36 and in the present analysis the number of items necessary to calculate a score was 5 out of 9 questions.

To assess disability level at study entry patients were divided into four groups: 1) no wheelchair or respiratory support, 2) only wheelchair, 3) only respiratory support and 4) both wheelchair and respiratory support. No division was made between partial and fulltime respiratory support, or whether it was invasive or non-invasive.

According to their nationality patients were divided into the following groups: Netherlands, United Kingdom, United States, Germany, Canada, Australia and other (Denmark, Austria, Switzerland, Spain, Italy, New Zealand, Greece, Taiwan and Luxembourg).

Statistical analysis for survival from diagnosis and from study entry
Survival was calculated from the date of diagnosis or study entry until the date of last follow-up, start of ERT or death. The survival times of patients who were alive at study end or lost to follow-up were censored. The survival times of the patients were also considered censored at the initiation of ERT.

For survival from diagnosis and from study entry, the influence on survival was tested for the following variables: gender, age at diagnosis, and nationality. The variables age at entry, disability level and RHS score were only tested for survival from study entry.

For survival from diagnosis, the PROC PHREG method in SAS was used, because most of our patients were enrolled months or years after diagnosis. This means that the data are 'left-truncated', as opposed to usual time-to-event data where all patients are followed from diagnosis. Estimates of survival from diagnosis in case not all patients enter the cohort study at the time of diagnosis require special calculations as described by Kurtzke.[21]

Univariate analysis for survival from study entry was estimated by using the Kaplan–Meier method. Factors influencing survival were identified with the log-rank test. Multivariate analysis was performed with the Cox proportional-hazards method.

Mortality of Dutch patients compared to the general population
Death probabilities from study entry were compared between the Dutch Pompe patients and the general population using death probabilities derived from the Dutch Central Bureau of Statistics (CBS).[22] For each case of our study population, the death probability per follow-up year of someone of the same age and gender from the general population was taken for comparison. Annual death probabilities per person were summed up and the sum of these cumulative death probabilities of the matched persons from the general population was used as the expected number of deaths. This was then compared to the observed number of deaths in our own cohort using the Poisson distribution.

All analyses were performed using SAS (version 9.2) or SPSS (version 15.0). Statistical significance was defined as a p-value ≤0.05 for all analyses.

RESULTS

Patient characteristics

As of December 2009, 303 adult patients were enrolled in the Pompe Survey. Thirty-five of them were excluded from the analyses. These were patients who had provided too little information about their diagnosis (n=8), patients with only baseline data available (n=15), patients already receiving ERT at study entry (n=2) and patients with important data missing such as date of birth (n=10). Thus, the current analyses covering the years 2002 to 2009 comprise a total of 268 adults with Pompe disease from 15 different countries. Patient characteristics are summarized in **Table I**.

Table I Patient characteristics of 268 untreated adult patients with Pompe disease

General characteristics	
Female, n (%)	141 (53)
Median age at study entry, years (range)	48 (19-79)
Median age at diagnosis, years (range)	38 (1-68)
Number of patients diagnosed in age categories of 15 years, n (%)	
<15 years	22 (8)
16-30 years	59 (22)
31-45 years	115 (43)
46-60 years	61 (23)
>61 years	11 (4)
Nationality, n (%)	
Netherlands	99 (37)
Germany	48 (18)
US	69 (26)
UK	20 (8)
Australia	13 (5)
Canada	9 (3)
Other	10 (4)
Median disease duration at entry, years (range)	9 (0-32)
Median follow up time, years (range)	3.5 (0.02-7)
Disability level at study entry, n (%)	
No wheelchair use or respiratory support[a]	127 (47)
Wheelchair use	34 (13)
Use of respiratory support	39 (15)
Both wheelchair use and respiratory support	68 (25)
Median RHS score* at study entry (range) (n=258)	27 (9-36)

Continuous variables are expressed as median (range). Categorical variables are expressed as n (%).
[a] 'Respiratory support' includes partial and fulltime, invasive and non-invasive respiratory support
* RHS assesses the level of participation/handicap; score varies between 9 (severe participation restrictions) and 36 (no participation restrictions).

At study entry patients' age varied between 19 and 79 years with a median age of 48 years. This did not differ significantly between countries. The median age at diagnosis was 38 (range 1-68) years. Median follow-up time from study entry was 3.5 years, with a maximum of 7 years. Seventy-eight percent of the patients were followed for 2 years or more and 62% of the patients for 3 years or more. Differences in disability level between countries were found, with the lowest rates of wheelchair and ventilator use in the Dutch patient group (32% and 26%, respectively). Almost all Dutch patients carried the most common *c.-32-13T >G (IVS1)* GAA mutation in combination with a fully deleterious mutation on the other allele. The *c.-32-13T>G (IVS1)* is a splice-site mutation leading to 10-20% residual activity of acid α-glucosidase and a broad clinical spectrum.[23]

Table II Characteristics of deceased patients (34 patients died in total, 23 of them prior to ERT)

	34 (All deceased patients)	23 (died prior to ERT)
General characteristics		
Female, n (%)	16 (47)	12 (52)
Median age at study entry, years (range)	54 (20-75)	51 (20-75)
Median age at death, years (range)	56 (23-78)	55 (23-77)
Median age at diagnosis, years (range)	42 (13-66)	42 (13-59)
Median disease duration, years (range)	14 (2-27)	16 (2-27)
Age at diagnosis in categories of 15 years, n (%)		
0-15 years	3 (9)	3 (13)
16-30 years	6 (18)	3 (13)
31-45 years	13 (38)	10 (44)
46-60 years	10 (29)	7 (30)
>61 years	2 (6)	0
Nationality, n (%)		
Netherlands	9 (27)	5 (22)
Germany	4 (12)	4 (17)
US	13 (38)	8 (35)
UK	4 (12)	3 (13)
Australia	1 (3)	0
Canada	2 (6)	2 (9)
Other	1 (3)	1 (4)
Disability level at study entry, n (%)		
No wheelchair use or respiratory support [a]	4 (12)	4 (17)
Wheelchair use	6 (18)	4 (17)
Use of respiratory support	7 (21)	4 (17)
Both wheelchair and respiratory support	17 (50)	11 (48)
Median RHS score* at study entry (range)	23 (9-36) (n=33)	22 (9-36)

Continuous variables are expressed as median (range). Categorical variables are expressed as n (%).
[a] *'Respiratory support' includes partial and fulltime, invasive and non-invasive respiratory support.*
* RHS assesses the level of participation/handicap; score varies between 9 (severe participation restrictions) and 36 (no participation restrictions.

Mortality

For 34 of the 268 patients, a death confirmation was received from the patient organiza-tion or the family. The median age at death was 56 years and did not differ significantly between countries. In 23 of the 34 cases information on cause of death was not available. For the Dutch patients (n=9), causes of death were reported as respiratory insufficiency (n=3), myocardial infarction (n=2), aortic dissection (n=1) and breast cancer (n=1). For two of them, cause of death was not known. Characteristics of the deceased patients are listed in **Table II**. Data from only 23 of the deceased patients (median age at death 55 years) could be used to estimate survival prior to ERT, since the other 11 received ERT prior to their death.

Survival from diagnosis

The estimated median survival after diagnosis -without enzyme replacement therapy-was 27 years. The estimated 5-year survival after diagnosis was 95%. At 10, 20 and 30 years this was 83, 65 and 40%, respectively (**Figure 1**).

Univariate and multivariate analysis showed that none of the tested factors were related to survival after diagnosis.

Because the time between the start of ERT and death was mostly less than one year and these patients were already severely affected when they started ERT, we performed a second analysis including all patients who died within 18 months after start of ERT (n=9). To be consistent with the deceased patients, the follow-up time for all other pa-tients on ERT was also extended by 18 months. In this analysis with 32 events, only age at diagnosis, accounting for gender and nationality, was related to survival (Hazard Ratio 1.55 per 10 years of age p<0.01).
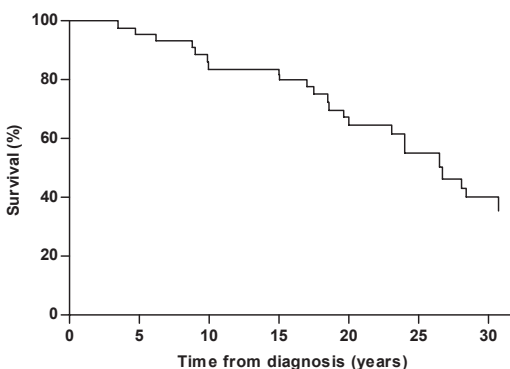
Figure 1 Survival estimates of 268 *untreated* adults with Pompe disease from diagnosis until end of study, start ERT or death. Twenty-three patients died during follow-up.

Survival from study entry

The Kaplan-Meier survival curve from study entry is shown in **Figure 2**. After 5 years of follow-up, 88% of the patients not yet receiving ERT were still alive.

Univariate analysis revealed a statistically significant difference in survival between groups based on disability level (overall p=0.002 log-rank, **Figure 3**), RHS score (overall p=0.002 log-rank, **Figure 4**) and age at study entry (overall p=0.03 log-rank). After five years 95% of patients without a wheelchair or respiratory support survived, while this was only 74% for patients with both wheelchair and respiratory support at study entry. **Table III** shows the 5-year survival with respect to potential prognostic factors.
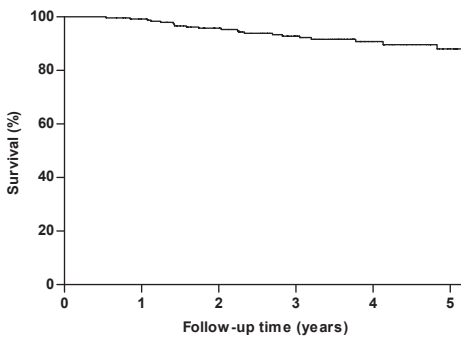
Figure 2 Kaplan Meier survival estimates of 268 *untreated* adults with Pompe disease from study entry until end of study, start of ERT or death. Twenty-three patients died during follow-up.
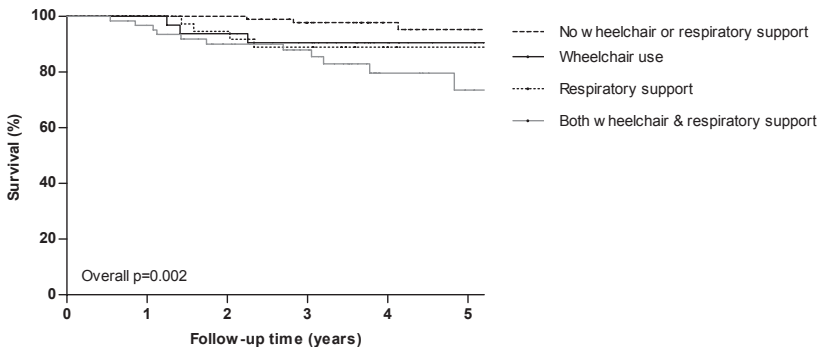
Figure 3 Kaplan Meier survival estimates of 268 *untreated* adults with Pompe disease from study entry until end of study, start of ERT or death by disability level. Twenty-three patients died during follow-up. 'Respiratory support' includes partial and fulltime, invasive and non-invasive respiratory support. P–value denotes result from log-rank test for trend.

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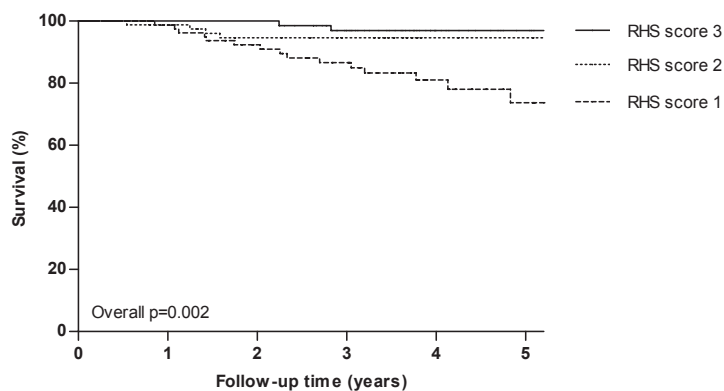
Figure 4 Kaplan Meier survival estimates of 268 *untreated* adults with Pompe disease from study entry until end of study, start of ERT or death by RHS score.
RHS score was divided into tertiles for comparison: RHS1= score <23, RHS2= score 23-30, and RHS3= score >30. Twenty-three patients died during follow-up. P–value denotes result from log-rank test for trend.

Multivariate analysis of the factors age at study entry, gender, nationality and disability level showed a significant effect of disability level (p=0.01), i.e. less disability at study entry was associated with better survival. Analyzing RHS score instead of disability level showed that a higher RHS score at study entry was also associated with better survival (p<0.001). In the analysis including 32 deceased patients, the factors age at study entry (p=0.01) and disability level (p=0.002) were significantly related to survival. When RHS score was analyzed instead of disability level both age (p=0.01) and RHS score (p<0.001) were significantly associated with survival.

Mortality of Dutch Pompe patients compared to the general Dutch population
For this part of the analyses 99 Dutch patients, with median age at entry 50 (range 24-79) years, were included. The Dutch subgroup included 5 deceased patients before start and 4 after start of ERT. The median age at death was 55 (range 41-78) years. Two of the patients who died after start of ERT had died during the first year of treatment; one other had died 18 months after start of ERT and one had started and stopped ERT in the year before death. These were all severely affected patients using a wheelchair and/or respiratory support before start of ERT. To compare the probability of death in the Dutch Pompe patient group with that in the general Dutch population two analyses were performed: one taking into account only the 5 deaths before start of ERT (median follow-up time 2.3 years) and one taking into account 9 deaths, while in the latter analysis extending the follow-up time after start of ERT with 1 year for every patient on ERT (median follow-up time 3.3 years). **Table IV** shows the results.

Table III Summaries of 5-year survival from study entry according to potential prognostic factors (23 deceased patients)

Prognostic factors	n	5-year survival percentages	p-value*
Gender			0.7
Women	141	86	
Men	127	91	
Age at diagnosis			0.4
0-15 years	22	81	
16-30 years	59	92	
31-45 years	115	89	
46-60 years	61	82	
>61 years	11	100	
Age at entry			0.03
18-30 years	32	91	
31-45 years	85	94	
46-60 years	104	89	
>61 years	47	77	
Nationality			0.7
Netherlands	99	90	
Germany	48	93	
US	69	85	
UK	20	67	
Other º	32	93	
Disability level at study entry			0.002
No wheelchair use or respiratory support ª	127	95	
Wheelchair use	34	91	
Use of respiratory support	39	89	
Both Wheelchair use and respiratory support	68	74	
RHS score at study entry*			0.002
1	85	74	
2	85	95	
3	88	97	

*Log rank test for the Kaplan-Meier curves, overall p-values of univariate analyses. º Due to small groups Canada and Australia were classified into the category "other". ª 'Respiratory support 'includes partial and fulltime, invasive and non-invasive respiratory support. * RHS score was divided into tertiles for comparison.1) < 23, 2) 23-30, 3)>30.

Table IV Mortality of 99 Dutch Pompe patients compared to general Dutch population

Analysis	Median follow-up time (range)	Observed no. deaths (O)	Expected no. deaths (E) *	Ratio (O/E)	p–value
1	2.3 (<1 month-7 years)	5	2.3	2.2	0.09
2	3.3 (<2 months-7 years)	9	2.8	3.2	0.002

Analysis 1: 5 deceased patients before start of ERT; **Analysis 2**: including 4 patients who died after start of ERT with follow-up of every patient on ERT extended with 1 year after start of ERT. * According to death probabilities derived from Dutch Central Bureau of Statistics.

DISCUSSION

Using data from the Pompe Survey, a long-term, disease-specific database using patient-reported outcome measures, we were able to perform the first study on survival and prognostic factors in adults with Pompe disease.

Over a prospective follow-up period of 7 years 34 of 268 patients died, 23 of them prior to ERT. Some of these patients died relatively young (23 years) and some reached very high ages despite Pompe disease (78 years). By using cumulative death probabilities of persons from the general population matched by age and gender, our study shows for the first time that mortality in adults with Pompe disease is higher than in the general population.

We also found that in our group of patients, diagnosed at a median age of 38 years, 17% died ten years after diagnosis. The median (50%) survival was estimated at 27 years after diagnosis. In an earlier study on the relation between disease severity and disease duration based on Pompe Survey data, we showed that 10-15 years after diagnosis 50% of the patients were wheelchair-bound or ventilator dependent.[24] Thus, although Pompe disease in adults generally manifests as a slowly progressive disorder, it seriously affects the lives of patients.

Several factors in our study had a significant effect on survival. For patients without a wheelchair or respiratory support the 5-year survival from entry was 95%, while for patients with a wheelchair and respiratory support this was 74%. In practice this means that patients with a wheelchair and/or respiratory support have a shorter life expectancy at any age compared to patients without wheelchair and respiratory support. RHS score at study entry, indicative of the level of handicap or participation, also was significantly associated with survival. Whether the RHS score is also useful as a prognostic tool in clinical practice is a topic for further investigation.

The strength of our study is its prospective design, the regular follow-up, the representation across countries, and the large sample size, especially for a rare disorder such as Pompe disease. In orphan diseases, it is quite unique to be able to gather information on a large group of patients over so many years, especially prior to therapeutic intervention. Our prospective data collection was achieved by relying on patient reported

outcome measures through a close collaboration with patient organizations. This approach enabled data collection without the support of a large physician's network that is – in orphan diseases – usually activated only after the introduction of a therapy. Our approach may stand model for data collection in other rare diseases. Since almost all newly diagnosed Pompe patients currently start with enzyme replacement therapy, this study might have been the very last chance to collect data on the natural course of Pompe disease.

Nevertheless, some methodological issues need further attention. Firstly, our patients were followed from 2002 onwards, which means that the majority entered the study at some cross-sectional point of their illness. The ideal method would have been to follow all patients from the time of disease onset or diagnosis until death. However, if we had applied those restrictions our study population would have been too small and the follow-up period would have been too short. Therefore, the next best thing was to also include the patients diagnosed before entering the study. This led to so-called 'left-truncated' data, with a grey area between diagnosis and study entry in which other patients may have died without entering our study, and could have caused an overestimation of the median survival. Additionally, because all data in the Pompe Survey are provided voluntarily, some deaths among enrolled patients who eventually became censored due to loss-to-follow-up (n=37) may not have been reported.

Second, differences in wheelchair and ventilator use were observed between countries, with the Dutch patients tending to be less severely affected on average. This may be explained by the fact that the Dutch group includes almost all patients known in the Netherlands, while the inclusion through patient organizations in the other countries may have led to a larger proportion of more severely affected patients. This may have affected the estimation of median survival time, but does not influence our main conclusions that mortality in adults with Pompe disease is higher compared to the general population and is associated with disease severity.

Furthermore, our patients were followed for up to 7 years, but median follow-up time was 3.5 years. Although a longer follow-up of untreated patients would offer more insight in their survival, such a study will be difficult to do as most patients currently receive ERT.

Because our aim was to investigate the natural course survival, we censored patients at the initiation of ERT. This means that 11 patients who died *after* start of ERT were not included as deceased patients in our initial analysis. Most (n=9) of these patients died within 1.5 year *after* start of ERT, or stopped ERT after a few infusions. As this treatment period is relatively short, we also performed analyses including these patients. Excluding these patients could have led to an underestimation of the number of deaths as these patients were already severely affected at the point they started ERT. All of them were

wheelchair-bound and/or used respiratory support and most probably would also have died without ERT. For the same reason, in our comparison of death probabilities between the Dutch subgroup and the general population we also included the 4 patients who died shortly after start of ERT.

Unfortunately, information on cause of death was lacking for the majority of the deceased patients. However in our study, mortality was compared with the data obtained from the Dutch Central Bureau of Statistics, which reports deaths irrespective of the cause. This comparison showed that the difference in mortality between the two groups was statistically significant. This in itself is important information, which can be used to evaluate the severity of disease and may serve as a reference when comparing the mortality of patients under treatment. With regard to the reported causes of death, it seems likely that death due to respiratory insufficiency is related to Pompe disease.[2, 25] Other causes, such as aortic dissection can also (in)directly be related to Pompe disease, as it may be a consequence of glycogen accumulation in vascular smooth muscle.[26]

Whether timely start of ERT can increase survival of adults with Pompe disease is currently unknown. In a recently published randomized controlled trial of alglucosidase alfa in late-onset Pompe disease, significant differences in walking distance and pulmonary function between the alglucosidase alfa and placebo groups were found.[16] Considering these results, and given the fact that most patients die of respiratory failure, it might be expected that ERT will also positively influence life expectancy. The present study, in which we show that Pompe disease has a serious negative impact on the life span of untreated adult patients, allows for future evaluation of the effect of ERT with respect to this important parameter.

CONCLUSION

Our study shows for the first time that mortality of untreated adults with Pompe disease is high compared to the general population. Both the need of a wheelchair and ventilator and a low RHS score are associated with higher mortality. Our results can serve as reference for future studies addressing survival of patients treated with ERT or alternative interventions. This information will also be valuable for families, genetic counsellors, and other health-care professionals when Pompe disease is diagnosed. Future studies should focus on identifying other factors – environmental or genetic – that may determine survival or disease progression in adults with Pompe disease, with or without treatment.

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CHAPTER 3.1

Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study

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ABSTRACT

Background Enzyme replacement therapy (ERT) in adults with Pompe disease, a progressive neuromuscular disorder, is of promising but variable efficacy. We investigated whether it alters the course of disease, and also identified potential prognostic factors.

Methods Patients in this open-label single-center study were treated biweekly with 20 mg/kg alglucosidase alfa. Muscle strength, muscle function, and pulmonary function were assessed every 3–6 months and analysed using repeated-measures ANOVA.

Results Sixty-nine patients (median age 52.1 years) were followed for a median of 23 months. Muscle strength increased after start of ERT (manual muscle testing 1.4 percentage points per year (pp/y); hand-held dynamometry 4.0 pp/y; both $p < 0.001$). Forced vital capacity (FVC) remained stable when measured in upright, but declined in supine position (–1.1 pp/y; $p = 0.03$). Muscle function did not improve in all patients (quick motor function test 0.7 pp/y; $p = 0.14$), but increased significantly in wheelchair-independent patients and those with mild and moderate muscle weakness.

Relative to the pre-treatment period (49 patients with 14 months pre-ERT and 22 months ERT median follow-up), ERT affected muscle strength positively (manual muscle testing +3.3 pp/y, $p < 0.001$ and hand-held dynamometry +7.9 pp/y, $p < 0.001$). Its effect on upright FVC was +1.8 pp/y ($p = 0.08$) and on supine FVC +0.8 ($p = 0.38$). Favourable prognostic factors were female gender for muscle strength, and younger age and better clinical status for supine FVC.

Conclusions We conclude that ERT positively alters the natural course of Pompe disease in adult patients; muscle strength increased and upright FVC stabilized. Functional outcome is probably best when ERT intervention is timely.

BACKGROUND

Pompe disease (OMIM number 232300) is an autosomal recessive metabolic myopathy caused by deficiency of the lysosomal enzyme acid α-glucosidase. This deficiency impairs lysosomal glycogen breakdown, leading to glycogen accumulation in several tissues.[1-4] The disease covers a broad clinical spectrum, ranging from a rapidly progressive infantile phenotype that results in death within the first year of life, to more slowly progressive forms in children and adults.[5-11] In adults, the disease generally presents as a limb-girdle myopathy. As well as the skeletal muscles, respiratory muscles – including the diaphragm – are affected.[1,12,13] As the disease progresses, most patients lose ambulation and require ventilatory support.[5,10,11,14,15]

Although Pompe disease used to be untreatable, patients' prospects changed in 2006 upon the introduction of enzyme replacement therapy (ERT) with recombinant human acid α-glucosidase. Initial studies in infants showed that ERT improved survival and motor outcome.[16-23] Several studies focusing on adult patients have been published since registration, but most report data in relatively small numbers of patients, or have a short follow-up.[24-29] Proof of efficacy was provided by the 18-month randomized-placebo controlled trial in 90 patients, 60 of whom received alglucosidase alfa. This showed that walking distance improved and pulmonary function in upright position stabilized.[29] As mild and severely affected patients had been excluded, the trial involved a selected group of patients.

The aims of the current study were therefore 1) to determine whether ERT alters the progressive course of Pompe disease in a broader adult patient population ranging from very severely affected to mildly affected; 2) to determine how much ERT alters the course of the disease relative to that reflected in pre-treatment data; and 3) to identify prognostic factors for response to treatment.

METHODS

Patients and study design

This single-center, prospective, open-label cohort study on the use of ERT was conducted from January 2005 to August 2009 at the Center for Lysosomal and Metabolic Diseases, Erasmus MC University Medical Center, Rotterdam, which is the Dutch national referral center for Pompe patients.

Patients were eligible for inclusion if 1) they were over 18 years of age; 2) their diagnosis had been confirmed by enzyme analysis in leukocytes or fibroblasts[30-32] and by mutation analysis[33]; 3) they had not previously been treated with recombinant human

acid α-glucosidase; 4) they had been treated for a minimum of 5 months; and 5) they were symptomatic, i.e. had measurable muscle weakness and/or diminished pulmonary function.

Patients received intravenous infusions with 20 mg/kg alglucosidase alfa every two weeks. Clinical assessments were performed every three to six months before the start of ERT and every three months thereafter.

The study protocol was approved by the Medical Ethical Committee at Erasmus MC University Medical Center. All patients provided written informed consent.

Twenty of the 69 patients participated in the randomized-placebo controlled trial on the safety and efficacy of alglucosidase alfa in late-onset Pompe disease; 13 in the treatment arm and seven in the placebo arm.[29] Data on FVC in upright position of these patients collected during the 18 months study period were included in the current analyses.

Skeletal muscle strength and function

Skeletal muscle strength was measured by manual muscle testing using the Medical Research Council (MRC) grading scale (range 0–5) and hand-held dynamometry (HHD) (Cytec dynamometer, Groningen, the Netherlands).[34,35] The following muscle groups were tested: neck extensors, neck flexors, shoulder abductors, elbow flexors, elbow extensors, hip flexors, hip abductors, knee flexors, and knee extensors. The MRC grade was also assessed for shoulder adductors, exorotators and endorotators, hip extensors, and hip adductors. A MRC sumscore was derived by adding the grades for all 26 muscles and expressing the sum as a percentage of the maximum possible score. HHD values (Newton) of each muscle group were first expressed as a percentage of the median strength of healthy males or females[35], and then combined into a sumscore by averaging these for all 16 muscle groups. If values for three or more muscle groups were missing, no sumscores were calculated for either method.

Muscle function was assessed using the Quick Motor Function Test (QMFT)[36]; this consists of 16 motor skills related to daily activities that require the use of muscles of the shoulder girdle, trunk, pelvic girdle and/or proximal lower limbs. Each item was scored on a 5-point ordinal scale, with 0 representing „cannot perform task" and 4 „can perform task with no effort". Adding all items gives a total score between 0 and 64. The actual sumscore was expressed as a percentage of the maximum score.

The use of wheelchair and walking aids was registered at each visit.

Pulmonary function

Forced vital capacity (FVC) in upright and supine positions was measured using spirometry as described earlier.[13,37] Results were expressed as a percentage of the predicted

normal value.[38,39] Values lower than 80% of predicted normal values were considered to be abnormal. The use of ventilatory support and hours of use per day was registered at each visit.

Safety assessments and laboratory investigations
Vital signs and adverse events were recorded at each visit. Electrocardiograms, a physical examination, and haematological, biochemical and urine analyses were made at regular intervals.

Statistical analysis
Longitudinal analysis of the outcome parameters (MRC, HHD and QMFT sumscores and FVC in upright and supine positions) was performed using repeated-measures ANOVA (random coefficient models). This method allows for irregular measurement times and different treatment durations. First, we assessed the mean annual change in the outcome parameters during the treatment period. Mean annual changes were expressed in absolute percentage points (pp/y). Analyses were also stratified by subgroups described in previous studies: gender, age (<45 years and ≥45 years old), disease duration (<15 years and ≥15 years), wheelchair use, ventilation use, FVC in upright position (≥80% and <80%) and MRC sumscore (in tertiles).[12,29]

Second, for the patients with pre-treatment and treatment data we established the extent to which the course of disease altered after starting ERT. To be included in this analysis, patients should have had at least one measurement done a minimum of 4 months before ERT. We included a linear effect of time before and during ERT in the repeated-measures ANOVA. Per individual, the two linear segments connect with each other at the time of start of ERT. This method is also known as the "broken-stick method" or as "piece-wise linear regression".

Third, we investigated prognostic factors for treatment response. As well as performing the subgroup analysis, we assessed the association between patients' characteristics and their individual response to ERT. Patients were classified into three groups: 1) non-responders, i.e. people in whom the course of disease (measured by one of the outcome parameters) was the same or worse during ERT than before; 2) good responders, whose disease course improved more than the median improvement of responders; and 3) moderate responders. Each patient's response was represented by the individual change in regression lines calculated in the "broken-stick" analysis. In patients without pre-treatment data, the natural course slope was imputed. Associations were tested using the Spearman test for continuous variables and the Chi-square trend test for categorical variables.

Analyses were performed with SPSS for Windows (version 17, SPSS Inc., Chicago, IL) or SAS (version 9.2, SAS Institute Inc., Cary, NC). A p-value lower than or equal to 0.05 (two-sided) was considered significant.

RESULTS

Patients

In total, 71 adult Pompe patients started ERT within the study period. Two were excluded because they had received ERT for less than five months: one died two months after starting ERT due to a dissection of the aortic arch; the second withdrew from the study after four months and died six months later due to respiratory insufficiency.

Table I shows the patients' characteristics at start of ERT. The median age at the start of ERT was 52.1 years, 52% were male, 40% used a wheelchair, and 37% used mechanical ventilation. The median treatment duration was 23 months (range 5–47 months). Of the 69 patients, 13 were treated for more than 3 years and six patients for less than 1 year, respectively for 5, 6, 7, 10, and two for 11 months.

All but one patient carried the common c.-32-13T>G (IVS1-13T>G) mutation on one allele in combination with another pathogenic mutation on the second allele. Of the patients carrying the c.-32-13T>G (IVS1-13T>G) mutation, 14 different mutations were found on the second allele. Forty-eight percent carried the c.525delT and 16% the c.2481+102_2646+31del mutation. The other mutations found were the c.-32-13T>G + c.1076-22T>G, c.1115A>T, c.1396G>T, c.1548G>A, c.172C>T, c.1799G>A, c.2314T>C, c.378_379del, c.461_469del, c.701C>A, c.896T>C and c.925G>A mutation. Of those mutations, 74% was indicated as very severe and 26% as potentially less severe (www.pompecenter.nl). For three patients the result of the mutation analysis could not be tracked, because these were performed by another center.

For 49 patients, pre-treatment data were available in addition to the treatment follow-up. These were slightly younger and less severely affected. Their median follow-up was 14 months before (range 4–33), and 22 months during ERT (range 6–41).

Table I Patients' characteristics and baseline values of clinical outcome measures at start of ERT

Characteristic	Total study population (n=69)	Patients with pre-ERT and ERT follow-up (n=49)	p-value [a]
Age at start of ERT in years, median (range)	52.1 (26.2–76.3)	50.1 (26.2–74.0)	0.83
Age at onset of symptoms in years, median (range)	30.8 (1.4–62.0)	32.0 (1.4–62.0)	0.63
Age at diagnosis in years, median (range)	39.6 (1.4–63.8)	40.9 (1.4–63.0)	0.97
Duration of disease at start of ERT in years, median (range)	9.3 (0.2–31.2)	8.3 (0.5–31.2)	0.74
MRC sumscore at start of ERT in percentage, median (range)	77.7 (48.3–92.3)	79.2 (60.8–92.3)	0.52
HHD sumscore at start of ERT in percentage, median (range)	69.3 (25.9–94.1)	69.3 (25.9–94.1)	0.97
QMFT score at start of ERT in percentage, median (range)	55.6 (10.0–89.1)	59.5 (21.7–89.1)	0.42
Time of mechanical ventilation at start of ERT per day in hours, median (range)	11.5 (0.0–24.0)	10.5 (0.0 –24.0)	0.81
FVC in upright position at start of ERT in percentage, median (range)	68.3 (11.3–106.9)	71.9 (26.5–106.9)	0.43
FVC in supine position at start of ERT in percentage, median (range)	46.8 (23.0–99.0)	52.4 (23.0–99.0)	0.71
Gender, No. of patients (%)			
- Male	36 (52)	21 (43)	0.43
- Female	33 (48)	28 (57)	
Wheelchair at start of ERT, No. of patients (%)			
- No wheelchair use	42 (61)	33 (67)	
- Partial wheelchair use	8 (12)	8 (16)	0.22
- Permanent wheelchair use	19 (28)	8 (16)	
Mechanical ventilation at start of ERT, No. of patients (%)			
- No mechanical ventilation	44 (64)	36 (74)	
- Non-invasive mechanical ventilation	19 (28)	10 (20)	0.72
- Invasive mechanical ventilation	6 (9)	3 (6)	

ERT=Enzyme Replacement Therapy, MRC sumscore=Medical Research Council sumscore, HHD sumscore=Hand-Held Dynamometry sumscore, QMFT score=Quick Motor Function Test score, FVC=Forced Vital Capacity, N/No.=Number of patients.
[a] Differences between the total group (N=69) and the group with pre-ERT and ERT follow-up (N=49) were calculated using Chi-square tests for the categorical variables, and using Mann-Whitney tests for continuous variables.

Skeletal muscle strength

During ERT, the MRC sumscore among all 69 patients rose by an average of 1.4 pp/y, and the HHD sumscore by 4.0 pp/y (both p<0.001; **Table II**). All individual muscle groups contributed to the effect (p≤0.02 for all muscle groups). Subgroup analyses showed that the mean annual increase in muscle strength during ERT was greater for women than for men (2.6 pp/y vs. 0.4 pp/y; difference between groups p<0.001 for MRC; and 6.3 pp/y vs. 2.0 pp/y, difference between groups p=0.05 for HHD). For the other subgroups investigated, there were no significant differences in the increase in muscle strength during ERT.

Before the 49 patients with pre-treatment and treatment data started ERT, their muscle-strength sumscores declined significantly (−1.2 pp/y for MRC, p=0.006; −2.8
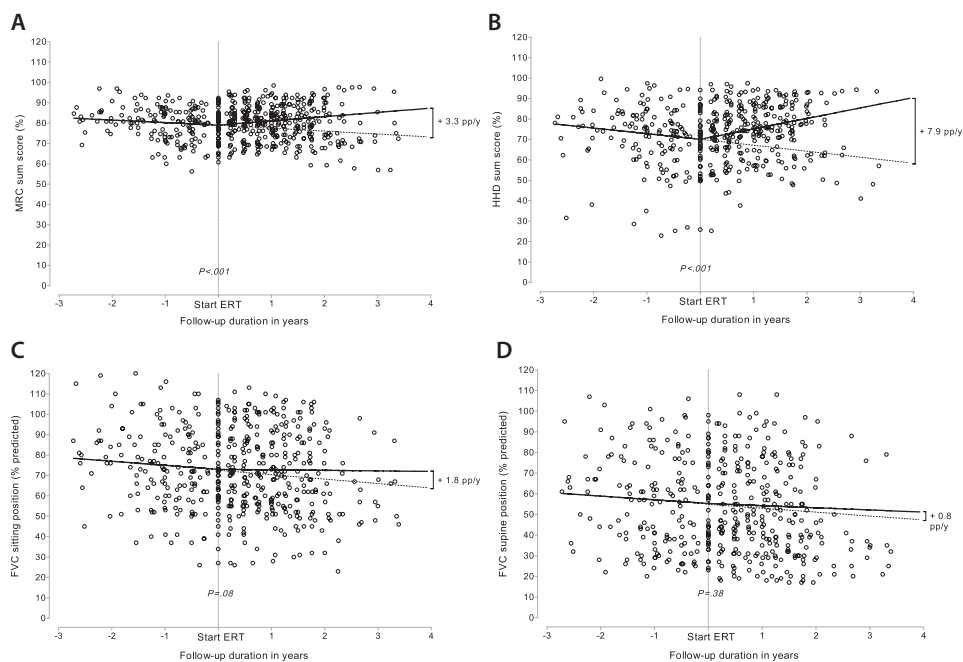
Figure 1 The clinical course of disease before and after the start of enzyme replacement therapy (ERT) in the 49 patients with ERT and pre-ERT follow-up data for **A**) MRC sumscore; **B**) hand-held dynamometry (HHD) sumscore; **C**) forced vital capacity (FVC) in upright position; and **D**) FVC in supine position. The figure shows all the individual observations for the different outcome measures (shown as dots) and the solid lines represent the estimated mean trend in these observations as calculated by the "broken stick" repeated-measures ANOVA. The dotted lines represent the extrapolated natural course slopes.

pp/y for HHD, p<0.001). Treatment data produced a significant improvement, the differences being +3.3 pp/y for the MRC sumscore and +7.9 pp/y for the HHD sumscore (both p<0.001; **Table II**, **Figure 1A** and **B**).

Skeletal muscle function

Although QMFT scores for all 69 patients increased by an average of 0.7 pp/y during ERT, this was not significant (p=0.14). Subgroup analyses showed that while muscle function improved in patients with mild muscle weakness (2.1 pp/y, p=0.01) and moderate muscle weakness (1.6 pp/y, p=0.05), it fell by 1.4 pp/y (p=0.08) in patients with severe muscle weakness (difference between groups p=0.004). In line with this, QMFT scores rose in wheelchair-independent patients (1.7 pp/y, p=0.008), but did not improve in those who were wheelchair-dependent (–0.6 pp/y, p=0.39; difference between groups p=0.02).

The number of patients who were partially or fully wheelchair dependent at the last follow-up visit was the same as at the start of ERT. Nevertheless, two of the 27 patients

Table II Clinical outcome measures during enzyme replacement therapy and relative to the natural course of disease

Clinical Outcome Measure	Natural course		Treatment course		Difference[a]	
	mean pp/y (95% CI)	p-value	mean pp/y (95% CI)	p-value	mean pp/y (95% CI)	p-value
MRC sumscore						
Total study population (N=69, M=558)			1.4 (0.8 to 2.1)	<0.001		
Patients with pre-ERT + ERT follow-up (N=49, M=523)	-1.2 (-2.1 to -0.4)	0.006	2.1 (1.2 to 3.0)	<0.001	3.3 (1.9 to 4.7)	<0.001
HHD sumscore						
Total study population (N=64, M=503)			4.0 (2.5 to 5.6)	<0.001		
Patients with pre-ERT + ERT follow-up (N=42, M=435)	-2.8 (-4.2 to -1.3)	<0.001	5.1 (3.0 to 7.3)	<0.001	7.9 (5.0 to 10.7)	<0.001
QMFT score						
Total study population (N=69, M=553)			0.7 (-0.2 to 1.7)	0.14		
FVC in upright position						
Total study population (N=62, M=475)			0.1 (-1.0 to 1.1)	0.92		
Patients with pre-ERT + ERT follow-up (N=46, M=480)	-2.0 (-3.1 to -0.8)	0.001	-0.2 (-1.6 to 1.2)	0.76	1.8 (-0.2 to 3.7)	0.08
FVC in supine position						
Total study population (N=54, M=411)			-1.1 (-2.1 to -0.1)	0.03		
Patients with pre-ERT + ERT follow-up (N=42, M=436)	-1.8 (-2.9 to -0.7)	0.002	-1.0 (-2.3 to 0.3)	0.12	0.8 (-0.9 to 2.4)	0.38

ERT=Enzyme Replacement Therapy, pp/y=percentage points per year, 95% CI=95% Confidence Interval, MRC sumscore=Medical Research Council sumscore, QMFT score=Quick Motor Function Test score, HHD sumscore=Hand-Held Dynamometry sumscore, FVC=Forced Vital Capacity, N=Number of patients, M=Number of Measurements. Data shown are mean changes in percentage points per year as calculated by repeated-measures ANOVA.
[a] Difference between the course of disease during treatment and the natural course.

who used walking aids at the start of ERT regained the ability to walk independently during ERT. Two other patients became dependent on walking aids.

Pulmonary function and use of mechanical ventilation
During treatment with ERT, FVC in upright position remained stable (0.1 pp/y, p=0.92), while FVC in supine position declined (−1.1, p=0.03) (**Table II**). Subgroup analyses showed that FVC in supine position did remain stable in patients under 45 years old (0.0 pp/y, p=1.0), but declined in those 45 years and over (−2.1 pp/y, p=0.002) (difference between groups p=0.03). There were no differences between subgroups for FVC in upright position.

Before start of ERT, FVC in upright and supine positions both declined significantly (−2.0 pp/y, p=0.001 and −1.8 pp/y, p=0.002, respectively). Compared to this, FVC in up-

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Table III Characteristics of individual response groups with regard to skeletal muscle strength and pulmonary function

	Non-responders	Moderate Responders	Good responders	p-value
Response groups for MRC sumscore, No. of patients (%)	**6 (9)**	**32 (46)**	**31 (45)**	
Female, No. of patients (%)	1 (17)	11 (34)	21 (68)	0.002
Age at start of ERT in years, median (range)	51.6 (38.0–66.2)	50.0 (26.2–67.0)	52.7 (29.2–76.3)	0.47
Disease duration at start of ERT in years, median (range)	12.1 (3.6–22.7)	11.1 (0.9–31.2)	9.0 (0.2–29.1)	0.55
MRC sumscore at start of ERT in percentage, median (range)	80.8 (60.8–86.9)	80.0 (48.3–91.5)	74.6 (53.3–92.3)	0.12
Wheelchair use at start of ERT, No. of patients (%)	2 (33)	9 (28)	16 (52)	0.11
FVC in upright position at start of ERT in percentage, median (range)	61.5 (34.4–79.3)	69.9 (11.3–105.0)	69.3 (16.4–106.9)	0.48
Ventilation use at start of ERT, No. of patients (%)	1 (17)	12 (38)	12 (39)	0.49
Response groups for FVC in upright position, No. of patients (%)	**9 (15)**	**26 (42)**	**27 (44)**	
Female, No. of patients (%)	5 (56%)	14 (54%)	13 (48%)	0.64
Age at start of ERT in years, median (range)	52.6 (31.9–69.2)	51.2 (35.7–71.7)	43.3 (26.2–76.3)	0.16
Disease duration at start of ERT in years, median (range)	8.3 (0.6–31.2)	14.1 (0.9–27.0)	4.7 (0.2–29.1)	0.06
MRC sumscore at start of ERT in percentage, median (range)	74.6 (63.1–91.5)	77.7 (48.3–90.0)	80.8 (67.5–92.3)	0.26
Wheelchair use at start of ERT, No. of patients (%)	4 (44%)	10 (38%)	6 (22%)	0.15
FVC in upright position at start of ERT in percentage, median (range)	66.6 (51.4–100.5)	68.3 (11.3–106.9)	69.9 (16.4–105.0)	0.95
Ventilation use at start of ERT, No. of patients (%)	2 (22)	8 (31)	8 (30)	0.76
Response groups for FVC in supine position, No. of patients (%)	**21 (39)**	**17 (31)**	**16 (30)**	
Female, No. of patients (%)	10 (48)	10 (59)	10 (63)	0.36
Age at start of ERT in years, median (range)	51.7 (31.9–67.0)	47.9 (26.2–62.9)	40.4 (29.2–74.0)	0.007
Disease duration at start of ERT in years, median (range)	10.7 (0.6–31.2)	4.7 (0.5–21.0)	5.0 (0.9–29.1)	0.12
MRC sumscore at start of ERT in percentage, median (range)	76.9 (60.8–91.5)	80.8 (69.2–90.8)	81.9 (69.2–92.3)	0.02
Wheelchair use at start of ERT, No. of patients (%)	8 (38)	2 (12)	2 (13)	0.05
FVC in upright position at start of ERT in percentage, median (range)	66.6 (45.4–106.9)	65.7 (41.5–105.8)	81.0 (62.6–105.0)	0.02
Ventilation use at start of ERT, No. of patients (%)	4 (19)	6 (35)	0 (0)	0.01

ERT=Enzyme Replacement Therapy, MRC sumscore=Medical Research Council sumscore, FVC=Forced Vital Capacity, No.=Number of patients.

right position improved during ERT, albeit at borderline statistical significance (+1.8 pp/y, p=0.08), while FVC in supine position did not (+0.8, p=0.38) (**Table II** and **Figure 1C** and **D**).

For the whole group there was no change in the median number of hours of ventilation per day between the start of ERT and the last treatment visit (Wilcoxon signed-rank test p=0.88). Mechanical ventilation time could be reduced by one or more hours per day in nine of the 25 patients who had been using mechanical ventilation at start of ERT. One of these was able to discontinue the use of his ventilator. In 14 patients, ventilation times remained the same, while ventilation was intensified in two. Nocturnal ventilation was initiated in two others.

Individual response with regard to skeletal muscle strength and pulmonary function
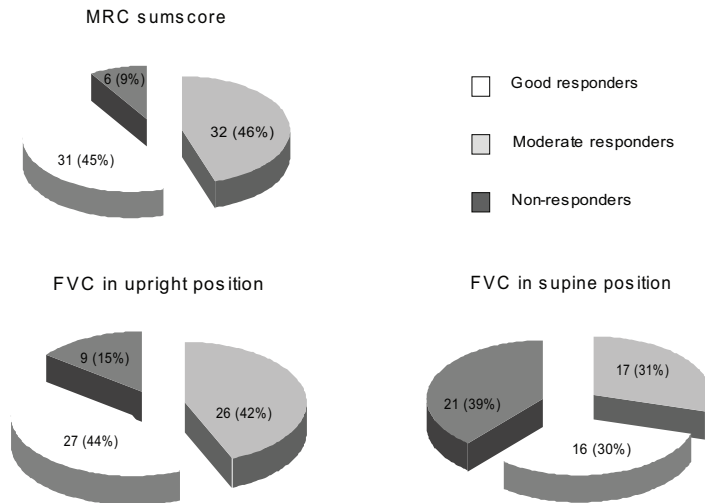
With regard to MRC sumscore, 31 patients (45%) were good responders, 32 (46%) moderate, and six (9%) non-responders (**Figure 2**). More women than men were good responders (p=0.002, **Table III**). Twenty-seven of the 62 patients with FVC data in upright position (44%) responded well, 26 (42%) moderately, and nine (15%) poorly. The characteristics between these FVC responder categories did not differ significantly.

Although FVC in supine position declined in the whole group during ERT, almost two-thirds of patients improved after starting ERT: 16 of 54 patients (30%) responded well, 17 (31%) moderately and 21 (39%) did not respond (**Figure 2**). Better responses were associated with younger age (p=0.007), less severe muscle weakness (p=0.02), wheelchair independence (p=0.05) and better pulmonary function in upright position (p=0.02) (**Table III**). None of the good responders were dependent on artificial ventilation.

Responses for muscle strength correlated poorly with responses for FVC. The correlation between response categories for FVC in upright and supine was moderate (Spearman's ρ 0.56, p<0.001).

Safety assessments and laboratory investigations

Laboratory safety parameters and ECGs remained unchanged during ERT. In total, 12 patients (17%) developed one or more infusion-associated reaction (IARs). These were similar to the IARs described in the randomized-placebo controlled study; most could be controlled by slowing infusion rates and/or giving premedication.[29] To prevent further IARs, seven patients received antihistamines as pre-medication, and five a combination of antihistamines and corticosteroids. At the end of the study three patients still had IARs, three patients were using antihistamines as premedication and two a combination of corticosteroids and antihistamines. Enzyme replacement therapy was discontinued in three patients who experienced IARs. In only one patient this was for safety reasons: this patient had a medical history of multiple auto-immune diseases and drug-induced

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MRC sumscore

6 (9%)

32 (46%)

31 (45%)

Good responders

Moderate responders

Non-responders

FVC in upright position

9 (15%)

26 (42%)

27 (44%)

FVC in supine position

17 (31%)

21 (39%)

16 (30%)

Figure 2 Individual response groups for skeletal muscle strength and pulmonary function
The number and percentage of good responders, moderate responders and non-responders are shown for
MRC sumscore, and forced vital capacity (FVC) in upright and supine positions.

allergies. In one of the other two patients IARs co-occurred with a very high antibody
titer and a poor response to ERT, as described previously.[40]

Two severely affected patients died. Their causes of death (sepsis after severe decubi-
tus and chronic respiratory insufficiency) were considered to be unrelated to ERT.

DISCUSSION

This study describes a large cohort of adult Pompe patients receiving treatment with
alglucosidase alfa. It reflects a unique situation in which most patients were also pro-
spectively followed before starting therapy, thereby extending median follow-up to 3
years (14 months before starting ERT and 23 months afterwards). We found that ERT sig-
nificantly altered the natural course of disease in adult Pompe patients. Muscle strength
increased significantly after they started ERT, and FVC in upright position stabilized. Even
though, at group level, FVC in supine position and muscle function did not improve dur-
ing ERT, there were improvements in certain subgroups of patients.

Like previous studies, we found that muscle strength deteriorated significantly before
the start of therapy.[10,11] However, the improvement in muscle strength after the start

of ERT was greater than that reported in other studies.[25-27,29,41] There may be various reasons for this. Our study was restricted to adult patients, but included patients across the entire disease spectrum. We tested more muscle groups, included more patients, and followed a longer treatment period than other studies, thereby producing over 500 measurements in total.

One new finding of this study is that women benefit more from ERT with respect to muscle strength than males. At the same dosage of 20 mg/kg bodyweight, it is possible that the relative dose of alglucosidase alfa received per gram of muscle-fiber tissue is higher in women than in men. Men generally have a higher lean body mass than women, and thus a somewhat higher muscle mass per kg.[42] As women also have smaller muscle fibers than men, they have a higher ratio of muscle-fiber surface to muscle-fiber volume. Consequently, they may have relatively more mannose 6-phosphate surface receptors, which mediate the uptake of alglucosidase alpha.[42,43] Other factors that may underlie the greater benefit women derive from ERT include muscle-fiber types, activity patterns, and hormonal influences.

Our study did not incorporate the six-minute walk test, a measure of functional endurance used in other studies[24,27,29] Instead, we assessed motor skills related to daily activities, using the QMFT, which was recently validated for use in patients with Pompe disease.[36] Although there was no change in muscle function across the entire group, there were significant improvements in wheelchair-independent patients and those with less pronounced muscle weakness. This finding indicates that timely intervention with ERT may be crucial to improving muscle function. The same is suggested by the results of the subgroup analysis in the trial of late-onset patients.[29]

The stabilization of FVC in upright position in our patients was similar to that recorded in the trial and in other studies[24,27,29]; the decline in FVC before ERT was similar to that observed in the placebo arm of the trial and natural course studies.[10-12,29]

This is the first study to report on the effect of ERT on the FVC in supine position. In the whole study population, this measure continued to deteriorate despite ERT, but individual results showed an improvement in almost two-thirds of patients. Patients were more likely to improve if they were younger, were independent of artificial ventilation, had a better FVC in upright position, and had less severe muscle weakness at the start of treatment. Again, this suggests that starting ERT early in the disease course may be beneficial.

Because ERT has been available since 2006, we performed an open-label study rather than a clinical trial, assessing the effect of ERT in all adult patients – from mild to severely affected – for many of whom we had also collected pre-treatment data prospectively. As this is an observational study, we could not correct for residual confounding. The small

sample size inherent to rare disorders meant that we could not apply a full multivariate model to identify prognostic factors.

CONCLUSIONS

In summary, by improving muscle strength and stabilizing pulmonary function in upright position, treatment with alglucosidase alfa positively altered the natural course of Pompe disease in adults. As well as finding that female gender is potentially a favourable prognostic factor for the effect of ERT on muscle strength, we found that younger age and better clinical status are favourable prognostic factors for pulmonary function. This suggests that it is important to start treatment early in the course of disease. Prognostic variables may help to identify patients with the best chances of benefiting from treatment.

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3.2

CHAPTER 3.2

High antibody titer in an adult with Pompe disease affects treatment with alglucosidase alfa

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ABSTRACT

Background Clinical trials have demonstrated beneficial effects of enzyme replacement therapy (ERT) with alglucosidase alfa in infants, children and adults with Pompe disease. Recent studies have shown that high antibody titers can occur in patients receiving ERT and counteract the effect of treatment. This particularly occurs in those patients with classic-infantile Pompe disease that do not produce any endogenous acid α-glucosidase (CRIM-negative). It is still unclear to what extent antibody formation affects the outcome of ERT in adults with residual enzyme activity.

Case We present the case of a patient with adult-onset Pompe disease. He was diagnosed at the age of 39 years by enzymatic testing (10.7% residual activity in fibroblasts) and DNA analysis (genotype: c.-32-13TNG/p.Trp516X). Infusion-associated reactions occurred during ERT and the patient's disease progressed. Concurrently, the antibody titer rose to a similarly high level as reported for some CRIM-negative patients with classic-infantile Pompe disease. Using newly developed immunologic-assays we could calculate that approximately 40% of the administered alglucosidase alfa was captured by circulating antibodies. Further, we could demonstrate that uptake of alglucosidase alfa by cultured fibroblasts was inhibited by admixture of the patient's serum.

Conclusion This case demonstrates that also patients with an appreciable amount of properly folded and catalytically active endogenous acid α-glucosidase can develop antibodies against alglucosidase alfa that affect the response to ERT.

INTRODUCTION

Pompe disease (glycogen storage disease type II, acid α-glucosidase deficiency, or acid maltase deficiency; OMIM 232300) was the first disease to be identified as a lysosomal storage disorder.[1] Acid α-glucosidase deficiency results in lysosomal accumulation of glycogen in all tissues, most notable in skeletal muscle.[2-4] Patients with a slowly progressive form of this disease typically present with skeletal muscle weakness in the limb-girdle region and/or respiratory insufficiency. Severely affected infants present as floppy babies due to generalized muscle weakness and have hypertrophic cardiomyopathy from birth on.[5,6]

Enzyme replacement therapy (ERT) has become available for the treatment of six lysosomal storage disorders; Gaucher disease, Fabry disease, MPS I, MPS II, MPS VI and Pompe disease. The registration of alglucosidase alfa (Myozyme®) for the treatment of Pompe disease dates from 2006.

Antibody formation against the administered enzyme is a well-known side effect of ERT.[7-11] In Gaucher disease about 15% of the patients start to produce antibodies within the first year of ERT while antibody formation is rarely associated with clinical deterioration.[7,8,12] In Fabry disease the majority of patients develop antibodies, but alarming effects have not been reported although the lowering of the plasma GL-3 level stalled in some sero-positive patients.[9] The majority of patients with these conditions become tolerant after prolonged treatment.

Compared to Gaucher and Fabry diseases the experience with antibody formation in Pompe disease is modest, but the currently available data indicate that the majority of Pompe patients on ERT develop antibodies against alglucosidase alfa.[13-15] A recent study clearly shows that infants who lack endogenous enzyme production (CRIM-negative patients) more readily develop high antibody titers than those who are CRIM-positive. Long-term studies (up to 3 years follow-up) suggest that sustained high antibody titers affect treatment outcome.[16]

Data on the long term effects of ERT in adults with Pompe disease are still scarce, but the first findings are hopeful in that ERT seems to halt disease progression and results in gain of muscle strength and pulmonary function in the best responders.[15,17-22] Thus far, it is unknown whether sustained antibody titers occur in adults with Pompe disease, who are all CRIM-positive, and whether high titers affect the response to treatment.

This case report concerns a 50-year-old male with adult-onset Pompe disease who started to experience infusion-associated reactions (IARs) during ERT with alglucosidase alfa. He had a continuous rise of antibody titer and his disease progressed. The aim of our study was to develop methods for measuring and evaluating the height of the immune response and its effect on treatment outcome.

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MATERIALS AND METHODS

Patient history

The male patient (patient 1 in this report; see **Table I**) presented at the age of 29 years with low back pain and a mild lumbar lordosis. The family history was negative for muscular and neurological diseases. Over the following decade his disease progressed. Bending over and climbing stairs became difficult due to a slowly progressive limb-girdle myopathy. He was diagnosed with Pompe disease at the age of 39 years. At the age of 49 years the patient was referred to our center (Erasmus MC, the Netherlands) and from then on was monitored twice yearly according to a protocol for standardized clinical follow-up of patients with Pompe disease not receiving ERT. One year later he was included in a randomized double blind placebo controlled clinical trial investigating the effects of alglucosidase alfa in late-onset forms of Pompe disease (LOTS; protocol number: AGLU02704/03206).[15] At the end of that trial it was disclosed that the patient had been assigned to the treatment group. He continued to receive ERT and underwent regular assessments while participating in a subsequent open-label study to secure standardized clinical follow-up.

Table I Baseline characteristics at start of ERT

	Patient 1	Patient 2	Patient 3	Patient 4
Sex	Male	Male	Female	Female
First symptoms (years)	29	46	36	44
Diagnosis (years)	39	52	62	51
Start ERT (years)	50	54	63	54
Walking aid	None	None	None	Walking stick
MRC sumscore (%)	85	92	82	75
Ventilation support	None	None	None	None
FVC sitting (%)	48	63	64	100
FVC supine (%)	24	33	NA	75
Creatine kinase U/l[a]	372	747	421	526
α-Glu activity fibroblasts[b]	9.4	7.6	16	13
α-Glu activity leucocytes[c]	2.9	8.2	0.1	0.5
GAA genotype	c.-32-13T>G/ p.Trp516X	c.-32-13T>G/ p.Gly309Arg	c.-32-13T>G/ p.Glu176fs	c.-32-13T>G/ p.Cys127fs

[a] *Creatine kinase U/l: control range 0–199 U/l;* [b] *Activities in fibroblasts are expressed as nmol 4 MU/mg h (control range: 40–180 nmol/mg h);* [c] *Activities in leucocytes are expressed as nmol glucose/mg h and were measured in the presence of 3 μM acarbose (control range: 48–215 nmol/mg h, n = 313).*

Reference patients

Three patients (patients 2, 3 and 4) were selected as reference subjects. They were matched for age, genotype, acid α-glucosidase activity, MRC sumscore and respiratory support at start of treatment (**Table I**). They also received alglucosidase alfa for more than one year, but did not experience infusion-associated reactions and responded well to ERT.

All patients described in this report have given informed consent for all studies they participated in.

Clinical assessments

Muscle strength was measured by Manual Muscle Testing (MMT) using the Medical Research Council (MRC) grading scale (range: 0–5; only full grades were used). A MRC sumscore was calculated for the following muscle groups: neck extensors, neck flexors, shoulder adductors, shoulder abductors, shoulder exorotators, shoulder endorotators, elbow flexors, elbow extensors, hip extensors, hip flexors, hip abductors, hip adductors, knee flexors and knee extensors. This sumscore was expressed as percentage of the maximum sumscore (range: 0–100%).

Muscle strength was also assessed by quantitative measurement of the maximum voluntary isometric contraction using Hand-Held Dynamometry (HHD)[23] and computerized quantitative muscle testing (QMT).[24,25] Nine muscle groups were assessed to calculate the HHD sumscore, which is expressed as percentage of the maximum sumscore. These muscle groups are the neck extensors, neck flexors, shoulder abductors, elbow flexors and elbow extensors, hip flexors, hip abductors, knee flexors and knee extensors. A composite QMT arm and leg score was calculated as previously described.[24]

The 6-minute walk test (6MWT) was a primary outcome measure in the LOTS and is a measure for the patient's performance. It measures the distance a patient can walk at his or her fastest pace in 6 min. The Forced Vital Capacity (FVC) was measured as pulmonary function test in sitting and supine position. Measurements were performed according to ATS/ERS standards[26] and reference values were derived from published data.[27-29] The results are expressed in percentage predicted FVC. All infusion-associated reactions (IARs), adverse events and serious adverse events were recorded.

Biochemical assays

The acid α-glucosidase activity in leucocytes[30] and fibroblast[31] was measured as previously described and is expressed in nmol/mg protein per hour. The protein concentration of cell homogenates was measured as described by van Diggelen, et al.[32]

The molecular forms of acid α-glucosidase derived from enzyme synthesis in fibroblasts were separated by SDS-PAGE (10% gel) and visualized by Western-blot analysis us-

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ing rabbit polyclonal antiserum raised against recombinant human acid α-glucosidase. Equal amounts of protein (40 µg) were applied in each lane. The ECL method (Amersham, GE-Healthcare) was used for the final staining.[33] The acid α-glucosidase specific protein species revealed at the end of this procedure are collectively called CRIM, which stands for Cross Reactive Immunologic Material.

Antibody titer

Blood samples for antibody titer determination were drawn just before start of alglucosidase alfa infusion. The serum was stored at –80 °C. Two-fold serial dilutions were made starting from 50 to 3200 fold in sodium-phosphate buffered saline (10 mM PBS, pH 7.0) containing bovine serum albumin (BSA, Sigma) in a concentration of 1 mg/ml (PBS/BSA). A 50 µl solution of alglucosidase alfa (total activity approximately 10 nmol 4 MU/h) in PBS/BSA was mixed with 10 µl diluted serum and 20 µl of a 1:1 suspension of Protein A sepharose CL-4B beads in PBS and incubated under continuous agitation for 1 h at room temperature. The beads were then removed by centrifugation (14,000 g) and the amount of antibody-bound alglucosidase alfa was calculated by measuring the enzyme activity in 10 µl of the supernatant using 20 µl 4-methylumbelliferyl-α-d-glucopyranoside (MUGlc, Sigma) substrate as described previously.

The antibody titer is expressed as the amount of alglucosidase alfa activity that is bound by the antibodies present in 1 µl of the patient's serum. Alternatively, the antibody titer was determined using an Enzyme-Linked ImmunoSorbent Assay (ELISA) and the results were confirmed by a Radio-Immuno Precipitation Assay (RIP) as described.[13].

Inhibition of enzymatic activity by antibody binding

Inhibition of enzymatic activity by antibody binding was studied by incubating 100 µl of a dilution of alglucosidase alfa in PBS/BSA (total activity of 20 nmol 4 MU/h) with 20 µl of two-fold serial dilutions of the patient's serum overnight at 4 °C. The next day the activity in the solution was measured with MUGlc and glycogen as substrates.

Pharmacokinetic analysis

Blood was drawn before the start of alglucosidase alfa infusion, 2 h after the start of infusion, 15 min before the end of the infusion, at the end of infusion, and at 15, 30, 60, and 120 min after the end of enzyme infusion. For patient 1, additional samples were drawn 4 h after the start of infusion and 3 h after the end of infusion. Plasma was separated and stored at –80 °C. Of a thousand-fold diluted plasma sample, 100 µl was incubated with 20 µl of either a suspension of sepharose beads or Protein A sepharose CL-4B beads. Further analysis was as described in the paragraph on assay of antibody titer.

Inhibition of alglucosidase alfa uptake

Two methods were used to evaluate whether the patient's antibodies interfere with up-take of alglucosidase alfa by human fibroblasts in culture. In the first method fibroblasts from a patient with classic-infantile Pompe disease, homozygous for the pathogenic sequence variation 525delT and fully deficient in acid α-glucosidase production, were seeded in a 6-well tissue-culture plate and maintained in 2 ml Ham's F10+ medium supplemented with heat inactivated fetal calf serum in a final concentration of 10%, at a temperature of 37 °C. For the purpose of measuring uptake of alglucosidase alfa overnight (16 h), the medium was buffered with 3 mM pipes to keep the pH slightly acidic at 6.8. Alglucosidase alfa was added in an amount equivalent to 2 µmol 4 MU/h. Patient's sera were added in a volume of 40 µl (final dilution 50 fold). The acid α-glucosidase activity in the medium was measured directly after enzyme addition and just before harvesting the fibroblasts. Uptake of enzyme by the fibroblasts was measured in a cell homogenate with MUGlc substrate, and the intracellular forms of the enzyme were visualized by SDS-PAGE followed by immunoblotting as described for the CRIM assay.

The second method involves flow cytometry, whereby human fibroblasts were grown in a 24-well tissue-culture plate overnight in Dulbecco's Modified Eagle's Medium. A known quantity of Oregon Green labelled alglucosidase alfa was pre-incubated with 2-fold serially diluted patient's sera and then added to the fibroblasts in HAMA uptake media for 3 h. The fibroblasts were harvested and the internalized labelled alglucosidase alfa was measured by flow cytometry. Percent inhibition was calculated by obtaining the Median Fluorescence Intensity (MFI) of the sample containing potential inhibitory antibodies, the MFI of the background, and the MFI of the sample containing only labelled alglucosidase alfa in 10% normal human serum.

% inhibition of enzyme uptake was calculated as follows:

$$\%\text{inhibition} = 100 - 100^* \ \frac{(\text{MFI sample} - \text{MFI background})}{(\text{MFI total} - \text{MFI background})}$$

Percentages of inhibition of enzyme uptake in normal sera and baseline samples of Pompe patients supported the use of 20% as cut-off point to define positive inhibitory antibody response and further verified the need to require this reactivity in at least two sera dilutions. Samples that have enzyme uptake inhibition greater than the assay cut-off are considered to be positive. The uptake inhibitory antibody titer is defined as the reciprocal of the highest serum dilution with a signal above the assay cut-off.

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RESULTS

Enzymatic and molecular diagnosis

The baseline characteristics of the patient discussed in this report (patient 1) and three reference cases (patients 2, 3 and 4) are presented in **Table I**. Patient 1 had an acid α-glucosidase activity of 9.4 nmol 4 MU/h mg protein in fibroblasts and 2.9 nmol 4 MU/h mg protein in leucocytes. DNA analysis revealed compound heterozygosity for c.-32-13T>G/p.Trp516X (**Table I**). c.-32-13T>G is the most common mutation among Caucasians with attenuated forms of Pompe disease and is characterized by the formation of only 10–20% correctly spliced GAA-mRNA and corresponding levels of acid α-glucosidase activity.[34,35] p.Trp516X (c.1548G>A) does not contribute to acid α-glucosidase production. These features stand out in **Figure 1** illustrating that patient 1 has much less mature acid α-glucosidase of 76 kDa and 70 kDa than a healthy individual, but substantially more than a selected case of classic-infantile Pompe disease without any acid α-glucosidase production.
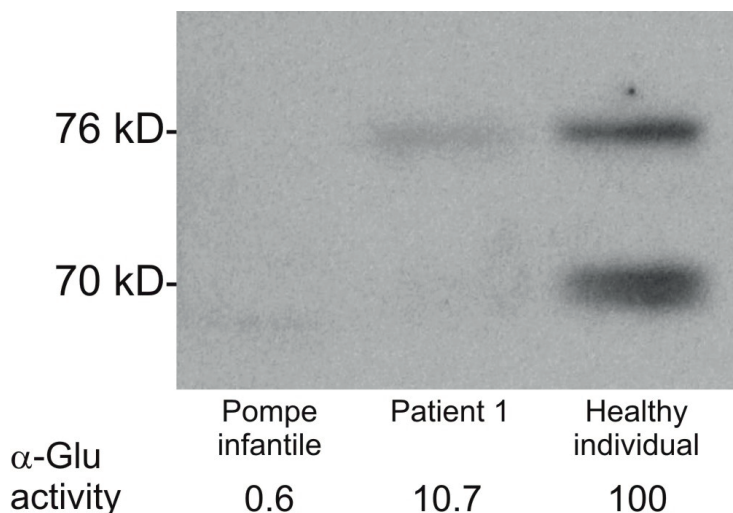
Figure 1 Acid α-glucosidase production in fibroblasts
Immuno-blot analysis of acid α-glucosidase production in fibroblasts of patient 1 compared with acid α-glucosidase production in fibroblasts of a patient with classic-infantile Pompe disease and a healthy individual. The acid α-glucosidase activities in patient 1 and in the patient with classic-infantile Pompe disease are expressed as percentage of the activity in the healthy individual. The activities were measured with MUGlc. Equal amounts of protein were applied to each lane (0.04 mg). The blot was stained as described in Materials and methods.
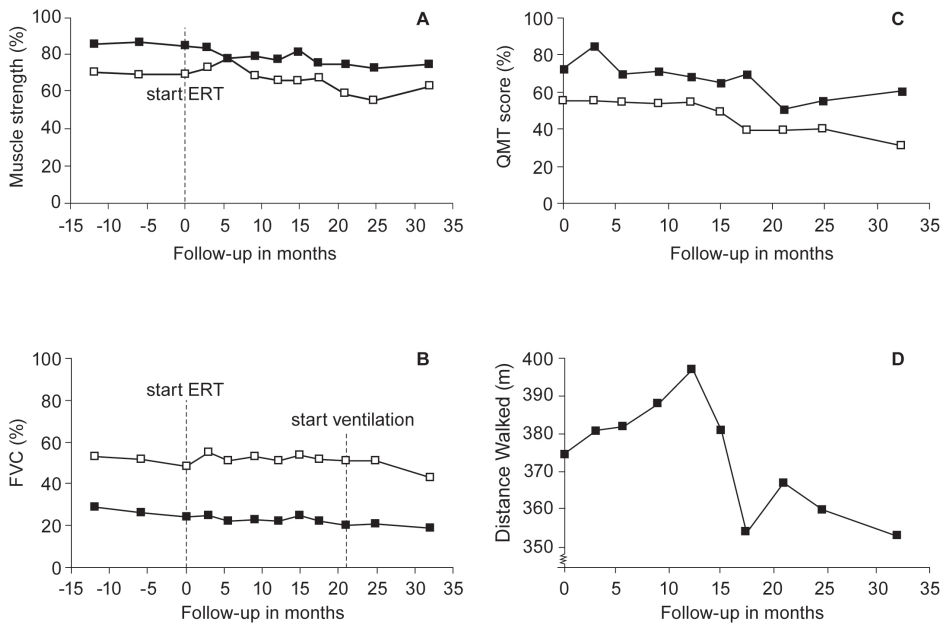
Figure 2 Muscle strength, pulmonary function and 6-minute walk test during ERT
A: Muscle strength as expressed in sumscore of nine muscle groups as measured with Hand-held dynamometry (□) and sumscore of 14 muscle groups as measured by MMT (■) in % of normal. **B**: Pulmonary function (FVC) as percentage of predicted in sitting (□) and supine (■) position. **C**: Muscle strength as measured with computerized quantitative muscle testing (QMT); for arms (□) and legs (■). **D**: Results of the 6-minute walk test as distance walked in meters (■).

Muscle strength, 6-minute walk test and pulmonary function

In the year before start of treatment the patient's condition gradually declined. With regard to muscle strength, the MRC sumscore was 86% at start of follow-up and the HHD sumscore was 70% (**Figure 2A**). Both sumscores declined with approximately 1% in the year before start of ERT. Pulmonary function measured as FVC in sitting and supine position declined with 5% during this year (FVCsitting from 53% to 48% and FVCsupine from 29% to 24% as shown in **Figure 2B**). Total follow-up after start of ERT with alglucosidase alfa was 33 months. After start of ERT, the disease continued to progress.

The FVCsitting and FVCsupine both declined with a rate of 1.9% per year (**Figure 2B**). After 21 months of treatment the patient became dependent on non-invasive nocturnal ventilation. In parallel, his muscle strength continued to decline during treatment. He experienced more difficulties with walking, fell more often, could not bend over, squat nor get out of his chair. The MRC sumscore declined with 3.7% and the HHD sumscore with 2.2% per year (**Figure 2A**). The QMT arm score declined with 4.5% and the leg score with 8.9% per year (**Figure 2C**). The distance walked as measured with the 6-minute walk

test (6MWT) initially increased, but started to decline after twelve months of treatment from 397 m to 353 m at 32 months of treatment (**Figure 2D**).

Infusion-associated reactions

Enzyme replacement therapy was initially administered according to the regular infusion schedule as shown in **Table II** with stepwise increase of the infusion rate. Infusion-associated reactions started to occur after five months of treatment. They were characterized by general malaise, shaking chills, and fever. The IARs could be managed with premedication (100 mg hydrocortisone), extending the duration of infusion steps I to III and lowering the infusion rate of step IV according to the adjusted infusion schedule shown in **Table II**. The IARs were endurable for the patient, but not fully controlled. Because of ongoing disease progression the treatment was finally stopped after a total duration of 33 months.

Table II Infusion schedules

	Step I	Step II	Step III	Step IV
Regular	5 ml/h	20 ml/h	88 ml/h	250 ml/h
	30 min	30 min	30 min	Till end infusion
Adjusted	5 ml/h	20 ml/h	88 ml/h	180 ml/h
	60 min	60 min	60 min	Till end infusion

Antibody formation and pharmacokinetic analysis

Since antibodies against recombinant forms of human acid α-glucosidase might elicit IARs and affect the therapeutic outcome, three types of tests were performed to evaluate antibody formation. **Figure 3** (dotted line) shows a continuous rise of antibody titer during the first 18 months of treatment as measured with ELISA.

A second antibody titer test was developed based on binding and precipitation of catalytically active alglucosidase alfa by antibodies in serum samples of the patient collected prior to enzyme infusions. **Figure 4** shows that alglucosidase alfa is precipitated by serially diluted serum of patient 1, whereas all activity remains in the supernatant when sera from patients 2, 3 and 4 are mixed in. The calculated titers at different time points are inserted in **Figure 3** (▲) as numbers to allow comparison with the ELISA assay. With both methods the highest titers were measured in month 18.

In month 32 after start of treatment, we measured the binding of circulating antibodies during infusion of alglucosidase alfa. **Figure 5** shows the total acid α-glucosidase activity in the plasma and the activity remaining after Protein A sepharose mediated immuno-precipitation of antibody-bound alglucosidase alfa. Two hours after the start of alglucosidase alfa infusion at low infusion rate, almost all acid α-glucosidase activity
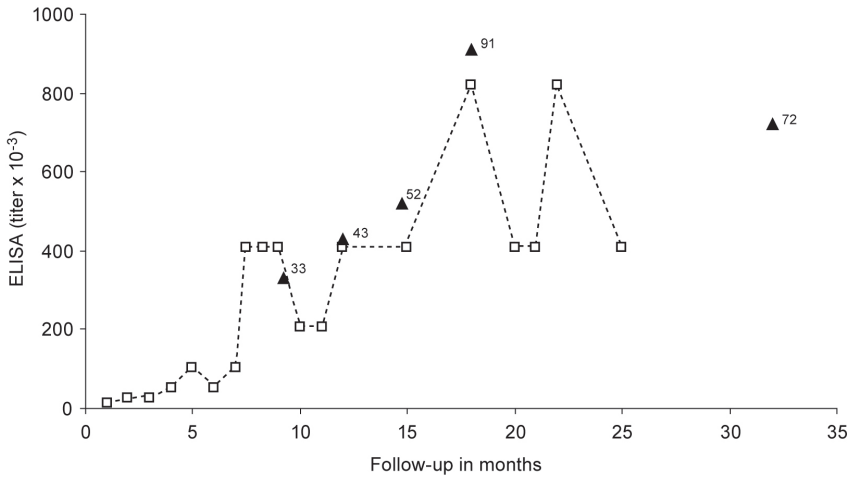
Figure 3 Antibody titer during ERT
Antibody titer as measured with ELISA (□ dotted line) and by immuno-precipitation (▲; in nmol 4 MU/h per 1 μl undiluted serum).
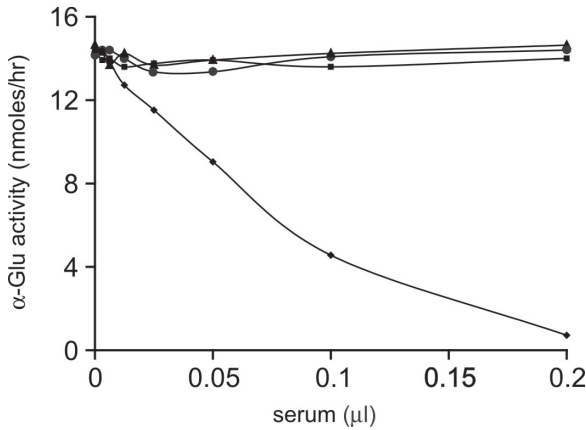
Figure 4 Antibody titration curves
Two-fold serially diluted sera of patient 1(♦), patient 2 (●), patient 3 (▲) and patient 4 (■), starting from a 50 fold dilution were added to a fixed amount of alglucosidase alfa. The antibody-bound alglucosidase alfa was precipitated with the aid of protein A sepharose, and the activity remaining in the supernatant measured with MUGlc.

is precipitated (**Figure 5A**). The proportion of antibody-bound enzyme decreases with increasing infusion duration and infusion rate and is 42% at the end of the infusion. **Figure 5B** shows the pharmacokinetics of three adult patients without IARs, without antibody titer, and with a good clinical response (patients 2, 3 and 4). In these three cases none of the infused alglucosidase alfa was antibody-bound.

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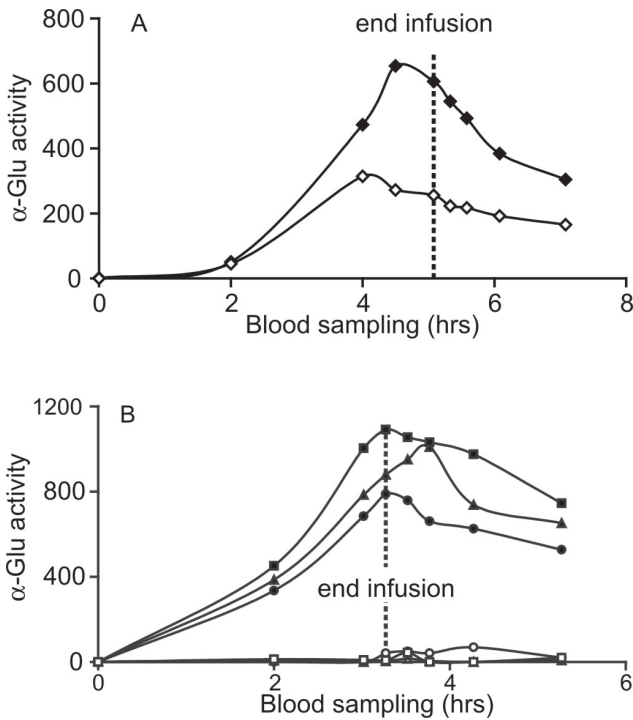
Figure 5 Antibody binding of alglucosidase alfa during infusion
Pharmacokinetic curves and the binding of infused alglucosidase alfa to circulating antibodies. Blood samples were collected just prior to start of infusion (0 h) and at regular time intervals thereafter. The closed symbols in panel A and B represent the total acid α-glucosidase activity in the plasma; the open symbols the amount of activity that is antibody-bound. The activity in the supernatant was measured with MUGlc and is in this figure expressed in nmol 4 MU liberated per 10 μl supernatant per hour. Panel A shows the pharmacokinetic curve of patient 1 (◆); panel B shows the pharmacokinetic curves of patient 2 (●), patient 3 (▲) and patient 4 (■).

In vitro uptake of alglucosidase alfa by fibroblasts

To investigate the adverse effect that antibody binding can have on the efficacy of ERT we applied two procedures to measure the uptake of alglucosidase alfa by in vitro cultured fibroblasts. In the first experiment we directly added alglucosidase alfa to the culture media and analyzed uptake in the presence of the sera of patient 1 with antibody titer (S1) and patient 2 without antibody titer (S2), and in the absence of human serum (S0). After 16 h of incubation, the acid α-glucosidase activity in the medium was in all cases less than added (1000 nmol 4 MU/ml), but on average 25% lower in S1 compared to S2 and S0 (**Figure 6**, medium). **Figure 6** also shows by Western-blot analysis that the enzyme added to the medium has a molecular mass of 110 kDa, which is characteristic for alglucosidase alfa. There was no obvious difference in the amount of enzyme-protein

kD	Medium			Cells			
110-							
95-							
76-							
	S0	**S1**	S2	S0	**S1**	S2	Inf Pompe
α-Glu activity	769	**564**	742	434	**181**	541	0.2

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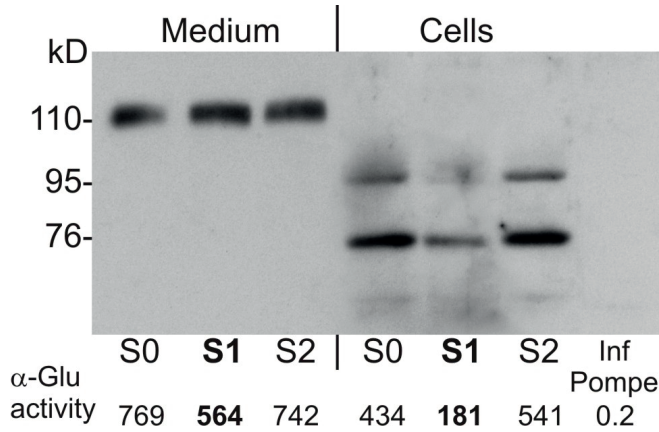
Figure 6 Uptake of alglucosidase alfa by fibroblasts
An amount of alglucosidase alfa equivalent to 978 nmol 4 MU/h was added to a 1 ml culture medium. The receiving fibroblasts were derived from a CRIM-negative patient with classic-infantile Pompe disease. The activity in the medium was measured the day after enzyme addition and is expressed in nmol 4 MU/h ml. The activity in the cells is expressed in nmol 4 MU/h mg cellular protein. The lanes contain 20 µl medium (Medium) or 0.04 mg protein (Cells). S0, no serum addition; S1, addition of serum from patient 1; S2, addition of serum from patient 2.

contained in the three media (samples S0–S2) despite the lower acid α-glucosidase activity in medium S1 (**Figure 6**, 564 versus 769–742). Thus, it seems that the serum of patient 1 has a slightly inhibitory effect on the catalytic function or stability of alglucosidase alfa. Part of the enzyme added to the fibroblasts was recovered inside the cells as shown in **Figure 6** (cells) by increased enzyme activity in the cell homogenates and the detection of processed forms of alglucosidase alfa (95 kDa and 76 kDa) by Western-blot analysis. Notably, in the three experiments that we performed there was repeatedly a clear difference in the amount of alglucosidase alfa inside the cells depending on the addition of serum from either patient 1 (S1) or patient 2 (S2), and no serum addition (S0). The lower protein signal in lane S1 compared to S0 and S2 indicates inhibition of enzyme uptake by the serum of patient 1 as is also reflected by the lower acid α-glucosidase activity in the fibroblasts fed with alglucosidase alfa in the presence of serum from patient 1 (**Figure 6**, cells).

In the second procedure the uptake of alglucosidase alfa by human fibroblasts was measured by flow cytometry. Oregon Green labelled alglucosidase alfa was incubated with two-fold serially diluted sera from the patient before addition to the cultured normal human fibroblasts. Percent inhibition was calculated and the end point inhibition titer was reported. **Figure 7** shows a continuous rise of the uptake inhibitory antibody
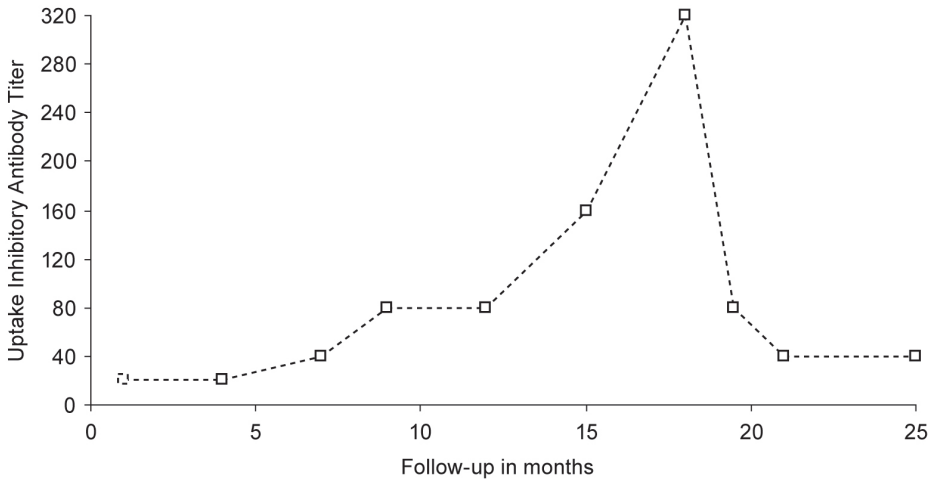
Figure 7 Uptake inhibitory antibody titers as measured by flow cytometry during ERT
Oregon Green labelled alglucosidase alfa was pre-incubated with 2-fold serially diluted patient's sera and then added to the fibroblasts. The titer was calculated as described in *Materials and methods*.

titer during the first 18 months of ERT followed by a decline. The peak titer was 320 at month 18.

DISCUSSION

Antibody formation is frequently encountered in patients who regularly receive protein infusions to combat the manifestations of a genetically determined protein deficiency. In Pompe disease, early anecdotal reports have mentioned the finding of high antibody titers in 2 of 3 children receiving the very first formulas of recombinant human acid α-glucosidase produced in CHO cells and suggested a correlation between antibody formation and CRIM status.[36] 'CRIM-positive' are the patients who produce a demonstrable amount of native enzyme and 'CRIM-negative' are those who don't. Another early study included one adult, two adolescents and four infants (one of which CRIM-negative) who were treated with recombinant human acid α-glucosidase from rabbit milk. The CRIM-positive adult patient in that study had the highest titer at the time of reporting.[37] A recent evaluation of all available data indicates that CRIM-negative patients are more prone to develop high antibody titers than CRIM-positive patients and that antibody formation negatively affects the treatment outcome in infants with Pompe disease.[16]

Our present study supports the notion that immune responses to alglucosidase alfa are not limited to infants but can also occur in adults despite the fact that they

Table III Response of patient 1 to ERT compared to the group-wise response to ERT

Outcome measure	Response of patient 1	Group-wise response (n = 60)
6MWT	−8.2[a]	16.8 (6.7 to 26.8)
QMT arm score	−4.5[b]	3.4 (1.3 to 5.5)
QMT leg score	−8.9[b]	0.8 (−0.7 to 2.3)
FVC sitting	−1.9[b]	0.8 (−0.1 to 1.7)

[a] *The figures represent the change from baseline in meters per year for the 6-minute walk test (6MWT) and* [b] *percentage points per year for QMT scores and FVC. The 95% confidence interval is given between brackets. The group-wise response per year was calculated from Table II of A. van der Ploeg, et al.*[15]

all are CRIM-positive; a situation comparable to type I Gaucher disease. In our case of adult-onset Pompe disease the poor response to treatment and the occurrence of IARs coincided with a very high antibody titer. The most alarming clinical finding was the ongoing decline of muscle strength of our patient during ERT compared to the positive group-wise response of 60 adult patients receiving ERT in a recently published randomized study of alglucosidase alfa in late-onset Pompe disease (summarized in **Table III**).[13-15]

The combination of sustained IARs and poor response to treatment was the main reason to investigate antibody formation as causative factor. The ELISA assay measuring the antibody titer is based on antibody binding to a fixed, but unknown, amount of immobilized alglucosidase alfa. The method employing Protein A sepharose beads measures the amount of catalytically active antibody-bound enzyme via precipitation. It is a semi-quantitative test showing how much enzyme is actually bound per serum volume. In case of patient 1, all alglucosidase alfa activity is ultimately precipitated at the highest serum volume (**Figure 4**). Inclusion of Protein A sepharose beads in the pharmacokinetic analysis provides an estimate of what percentage of administered enzyme is captured by the patient's circulating antibodies. The results obtained with the different methods corroborate each other. ELISA shows a gradual rise of antibody titer in patient 1 from start of treatment. The antibody titer test employing Protein A sepharose beads shows a similar trend (**Figure 3**) and additionally reveals the potential impact of a high titer on ERT as illustrated by the following calculation. The specific activity of alglucosidase alfa is approximately 300 μmol 4 MU/h mg protein. A titer of 91 nmol 4 MU/h μl (highest titer in **Figure 3**) implies that 1 μl of the patient's serum contains enough antibodies to bind 3×10^{-4} mg alglucosidase alfa. Thus, the antibodies in 2 liter serum can capture 600 mg alglucosidase alfa while a patient with a weight of 80 kg receives a total dosage of 1600 mg. It is evident that a substantial part of the total infusion dosage is bound by antibodies (37.5% in this example). The pharmacokinetic analysis including Protein A sepharose beads leads to a similar conclusion (42% at end of infusion; **Figure 5A**). **Figure 3** shows that an ELISA titer of 400,000 to 800,000 roughly corresponds to a titer of 50 to 90 nmol 4 MU/h μl. If one translates these figures to the situation in infants receiving ERT, the circulating antibodies capture more than half of the alglucosidase alfa dosage

that is administered to infants with equally high antibody titers. This would provide an explanation for their poor response.[16]

The way antibody binding affects ERT is not totally clear, but from the experiments we performed it is evident that admixture of antibodies to alglucosidase alfa inhibits the uptake of the enzyme by fibroblasts in a cell culture system. Kishnani et al. and Nicolino et al. in their recent publications stated that some of their patients also produced antibodies that interfered with the uptake of alglucosidase alfa.[14,16] The most likely hypothesis for the poor response of our patient is that antibody binding also inhibits the in vivo uptake of alglucosidase alfa by the target tissues. The same adverse effect of antibody formation on enzyme uptake has previously been demonstrated in relation to the treatment of patients with Fabry disease. In that study, the inhibitory effect was not only demonstrated in vitro but also in vivo by injecting mice.[38]

Antibody binding and immune-complex formation can also inactivate the enzyme's catalytic function, but that does not seem to be the case in this patient. This holds for the activities towards both glycogen and MUGlc (results not shown). Frequently, patients receiving ERT become tolerant after prolonged treatment. If this is not the case immune tolerization protocols may help to annihilate the detrimental effects of antibody formation. One report on the successful application of immune tolerization in a case of CRIM-negative infantile-onset Pompe disease has recently been published.[39]

It remains enigmatic why the patient in our study developed exceptionally high antibody titers since he is CRIM-positive and has the most common GAA mutation (c.-32-13T>G). The immune response in this case is apparently also determined by genetic background or environmental factors.

Conclusively, we have shown that alglucosidase alfa can occasionally elicit an adverse immune response even in CRIM-positive adult patients. A high antibody titer can become detrimental to the therapeutic effect of ERT when the number of circulating antibodies approaches the number of administered alglucosidase alfa molecules. The antibody titer assay and the pharmacokinetic analysis, as described in this report, including precipitation of antibody-bound alglucosidase alfa, give a meaning to the height of the antibody titer. If the circulating antibodies inhibit uptake of enzyme by the target tissues, they reduce the effective dosage of alglucosidase alfa. Immune-surveillance should be a routine procedure in the follow-up of patients receiving ERT for Pompe disease independent of the patient's clinical subtype or CRIM status.

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Chapter

3.2

3.3

CHAPTER 3.3

Effects of antibody formation during enzyme replacement therapy in Pompe disease: results of three year follow-up in 73 adult patients

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ABSTRACT

Purpose High sustained antibodies against enzyme replacement therapy with alglucosidase alfa adversely affect treatment outcome in classic-infantile Pompe disease. The long-term effects of antibody formation on therapeutic efficacy in other forms of Pompe disease are unknown.

Methods This prospective study included 73 adult patients treated with 20 mg/kg alglucosidase alfa every other week for 3 years. Antibodies were measured using ELISA before start of ERT and during ERT at 6, 12 and 36 months. Their effects on enzyme activity were measured *in vitro*. Clinical parameters included muscle strength, pulmonary function, and infusion-associated reactions.

Results 71 of the 73 (97%) patients developed antibodies. At 6 months of ERT, we found the highest titer, as the geometric mean titer was 1:1259 (95% CI 1:696 to 1:2276). Thereafter, the titer declined to 1:270 after 36 months of treatment. Individual titers and titer courses varied, but in the majority (93%) of patients titers decreased or stabilized with continued enzyme replacement therapy. Patients with high maximal titers showed moderate to high levels of enzyme inhibition *in vitro*. The height of the antibody titers correlated with the occurrence of infusion-associated reactions. At group-level no poorer clinical outcome was found as assessed by MRC sumscore and FVC in upright and supine positions. The exception was the patient with the highest antibody titer (1:3,906,250), who showed an ongoing clinical decline during ERT.

Conclusion Antibody formation to alglucosidase alfa in adults with Pompe disease increases the risk of infusion-associated reactions, but is not associated with unfavorable clinical outcome at group-level during 3 years of treatment.

INTRODUCTION

Pompe disease, also known as acid maltase deficiency and glycogen storage disease type II (OMIM 232300), is an autosomal recessive lysosomal storage disorder due to a deficiency of the enzyme acid α-glucosidase (GAA).[1, 2] This results in intralysosomal accumulation of glycogen in all tissues, but most notably in skeletal muscles.

Since 2006 enzyme replacement therapy (ERT) with alglucosidase alfa (recombinant human acid α-glucosidase, rhGAA, Myozyme®) is available for patients with Pompe disease. Registration was mainly based on studies performed in classic infantile patients – the most severely affected patients – suffering from rapidly progressive muscle weakness and a hypertrophic cardiomyopathy.[3, 4] Treatment with ERT leads to substantial improvement in survival, motor outcome and reversal of the hypertrophic cardiomyopathy,[5-9] whereas untreated infants succumb to cardiorespiratory failure within the first year of life.[3, 4]

Children, juveniles and adults exhibit a milder phenotype (non-classic Pompe disease) and suffer from gradually progressive limb-girdle muscular weakness and respiratory insufficiency.[10-14] The first symptoms can present at any age, from early infancy to late adulthood. The natural course of the disease is more heterogeneous as compared to classic-infantile Pompe disease, but over time the majority of patients becomes wheelchair and ventilator dependent.[15, 16] Subsequent studies, including a randomized-clinical trial, have also demonstrated positive effects of ERT on muscle strength, pulmonary function and survival in non-classic Pompe disease.[17-23] While the majority of patients benefits from ERT, profound variations in individual treatment responses have been observed in all forms of the disease.[5-9, 18, 19, 21-24]

Several factors are known to influence the response to ERT in classic-infantile Pompe disease. For example, an early start of treatment within the first weeks after birth and a relatively good patient's condition are both associated with a better clinical outcome.[8, 9, 25] However, high sustained anti-rhGAA antibodies can be associated with a less favorable response to ERT.[26, 27] These high sustained titers occur more frequently in patients with a CRIM (cross-reactive immunologic material) negative status, i.e. those patients who produce no endogenous GAA at all.[27, 28]

In children and adults with Pompe disease predictive factors with regard to treatment outcome are less well established. Younger age and better clinical status at start of ERT seem to be favorable prognostic factors for the effect of ERT.[19, 23, 24] So far, it remains unclear whether antibody formation in these patients is of influence on treatment efficacy. Patients with non-classic Pompe disease synthesize a fair amount of catalytically active GAA themselves. Hence, it was anticipated that their immune responses would be less vigorous and would not adversely affect treatment outcome. This assumption was

endorsed by the only randomized controlled trial for ERT, the Late-Onset Treatment Study (LOTS).[23] In this study all of the 59 adult patients receiving alglucosidase alfa developed anti-rhGAA antibodies during 18 months of treatment, but not to an extend that the effect of treatment was impaired. One patient in this trial had a very high and sustained antibody titer (maximal titer of 1:819,200). Extended follow-up (up to 33 months ERT) of this patient showed declining muscle strength, progressive respiratory insufficiency and persisting infusion-associated reactions (IARs).[29] Pharmacokinetic studies demonstrated that the patient's anti-rhGAA antibodies captured approximately 40% of administered alglucosidase alfa and *in vitro* studies showed inhibition of enzyme activity and enzyme uptake by cultured fibroblasts. A following publication by Patel *et al.* described three cases with adult-onset Pompe disease who all developed high sustained antibodies coinciding with a poor clinical response to ERT,[30] suggesting that undesirable effects of antibodies may also affect the treatment response of patients with non-classic Pompe disease. Because the impact of antibody formation on clinical outcome in non-classic Pompe disease is not fully understood, and as the disease is characterized by a slow disease progression, longer follow-up studies in large patient cohorts are desired to further investigate this point.

For these reasons we studied anti-rhGAA antibody formation in a cohort of 73 adult Pompe patients over a treatment period of 36 months. Clinical parameters included IARs, skeletal muscle strength and pulmonary function in upright and supine positions. This study represents the largest cohort with the longest follow-up for the effects of antibody formation on therapeutic efficacy of alglucosidase alfa and association with occurrence of IARs in adults with Pompe disease to date.

PATIENTS AND METHODS

Patients and Study Design
In 2005 the ongoing single-center, prospective, open-label cohort study on the natural disease course and the use of ERT in adults with Pompe disease was initiated at the Center for Lysosomal and Metabolic Diseases at the Erasmus MC University Medical Center (the designated center of expertise for Pompe disease in the Netherlands).
Patients were eligible for inclusion in the study if 1) they were over 18 years of age; 2) their diagnosis had been confirmed by enzyme analysis in leukocytes or fibroblasts[31-33]; 3) they had not previously been treated with rhGAA; and 4) they were symptomatic, i.e. had measurable muscle weakness and/or diminished pulmonary function. In this study on anti-rhGAA antibody formation, patients were included who started treatment between January 2005 and July 2010 (to allow a three year treatment period) and for

whom blood samples were available just before start of ERT and during ERT at 6, 12 and approximately 36 months. Data presented here were collected from January 2005 up to February 2013. During the study all patients intravenously received 20 mg/kg alglucosidase alfa every other week. The study protocol is described in more detail elsewere.[19]

The Institutional Review Board approved the study protocol, and all patients provided written informed consent.

Antibody Detection

Blood samples were either drawn just before the infusions, or, in case patients received home treatment, on a day patients did not receive ERT. When a blood sample was missing at 36 months ERT, a sample between 24 and 42 months was used closest in time to 36 months. Blood samples were stored at -20 $^{\circ}$C. Enzyme-linked immunosorbent assays (ELISA) were performed as described.[34] Experiments were repeated two to four times for high antibody titers ($\geq$1:31,250).

For twelve patients, titers were determined in the 6 and 12 months sera as part of the LOTS study using a non-commercial ELISA assay established in the Genzyme Clinical Specialty Laboratory (Genzyme Corp., Framingham, Massachusetts).[23, 35] For four of these, 6 and 12 months sera were used to validate the Erasmus MC ELISA protocol. Exact titers differed due to different dilution ranges (5 x in the Erasmus MC assay, 2 x in the Genzyme assay) and due to higher dynamic range of the Erasmus MC assay, but in all cases, the patients were placed in identical titer groups (no-low, intermediate, or high). Average values were used in these cases. For reasons of graphical representation of **Figure 1B**, titers derived from 2-fold dilutions were rounded to titers derived from 5 fold dilution.

Effects of antibodies on alglucosidase alfa activity

Fibroblasts from a patient homozygous for the 525delT pathogenic mutation and therefore fully deficient for endogenous GAA activity were grown in 24-well tissue-culture plates and kept overnight at 37 $^{\circ}$C 24 hours in Ham's F10+ medium supplemented with 10% heat inactivated fetal calf serum (FCS) and antibiotics. The medium was replaced with 200 μl HAM's F10+ medium containing antibiotics, 3 mM PIPES, alglucosidase alfa (in an amount equivalent to 200 nmol GAA activity), and 20 μl of the patients' sera. FCS served as control. Fibroblasts and medium were harvested after 24 hours. Alglucosidase alfa activity was measured using 4-methylumbelliferyl α-D-glucoside (4MU) as substrate as described.[34] The experiment was repeated three times for patients with an antibody titer and twice for patients without a titer. All measurements were performed in duplicate. Alglucosidase alfa activity was expressed as percentage of the activity present in FCS, measured in the medium directly after addition of alglucosidase alfa.
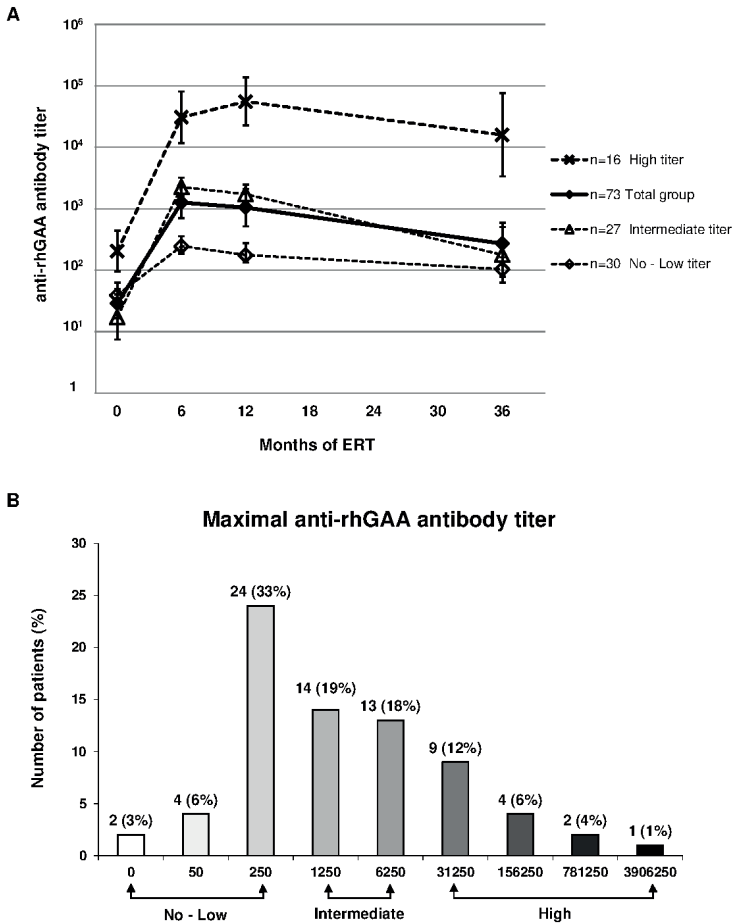
Figure 1 Anti-rhGAA antibody titer course and maximal titers during 36 months of enzyme replacement therapy
A Geometric mean and 95% confidence intervals of anti-rhGAA antibody titer course during enzyme replacement therapy for the total group and separately for patients with high titers, intermediate titers and no-low titers. **B** Distribution of the maximal anti-rhGAA antibody titers. Based on the highest antibody titer measured, patients were divided into the following groups: 1) no or low titer (0 – <1:1250); 2) intermediate titer (1:1250 – <1:31,250); and 3) high titer (≥1:31,250).

Clinical Outcome Measures and Infusion-Associated Reactions

Skeletal muscle strength and pulmonary function tests were performed every three to six months before the start of ERT and every three months thereafter. Methods are described in more detail elsewhere.[19] Briefly, skeletal muscle strength was assessed using manual muscle testing according to the Medical Research Council (MRC) grading scale. A

sumscore was calculated combining scores of the most affected muscles and the calculated score was expressed as a percentage of the maximum possible score. Forced vital capacity (FVC) was measured in upright and supine positions. Results were expressed as a percentage of predicted normal values. All clinical data up to a follow-up of 42 months ERT were incorporated in the analysis, as this was the maximal allowed follow-up for inclusion of blood samples for antibody testing.

Vital signs, IARs and medical interventions to treat IARs were recorded every other week when patients received their alglucosidase alfa infusions. We defined IARs as symptoms or signs with onset during the infusion or within a 48 hour time frame after start of infusion. Only IARs that were clinically judged to have a possible, probable, or definite relationship with the infusion were included in the analysis. The severity of IARs was classified as mild, moderate, or severe.

Statistical Analysis

Patients were divided into groups according to the highest titer observed: 1) no or low titer (0 – <1:1250); 2) intermediate titer (1:1250 – <1:31,250); and 3) high titer (≥1:31,250). When differences in demographic and clinical characteristics were calculated, it concerned the variables as shown in **Table I**. To calculate differences across the three antibody titer groups, we used the chi-square trend test for nominal data and the Kruskal-Wallis test for continuous and ordinal data.

To estimate whether there was an effect of antibody formation on treatment outcome as assessed by MRC sumscore and FVC in upright and supine positions, we carried out the following statistical analysis. First, in the total group, repeated measures ANOVA applying a piecewise regression ('brokenstick' method) was used to calculate: 1) the mean pre-treatment course; 2) the mean treatment course at 36 months ERT; and 3) the response to ERT at 36 months ERT. The latter is defined as the difference in course between the pre-treatment and the treatment period. As the mean treatment course in MRC sumscore violated the assumptions of linear regression, an exponential regression model was fitted. For the reason that the duration of follow-up for the pre-treatment course differed among patients, it was expressed in absolute percentage points per year (pp/y). The treatment course and response to ERT were expressed in percentage points (pp) over a 36 months treatment period.

Lastly, to estimate whether there were differences in pre-treatment course, treatment course, and response to ERT across the three antibody titer groups, a subgroup analysis was performed applying the same statistical methods as in the total group.

Analyses were performed with SPSS for Windows (version 21, SPSS Inc., Chicago, IL) and (repeated measures analysis using) SAS (version 9.3, SAS Institute Inc., Cary, NC). The

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Table I Patients' characteristics at start of enzyme therapy for the total group and antibody titer groups

Characteristic	Total population n = 73	No – low titer n= 30	Intermediate titer n=27	High titer n=16	p-value
Gender – No. of patients (%)					
- Male	37 (51)	16 (53)	13 (48)	8 (50)	0.79
- Female	36 (49)	14 (47)	14 (52)	8 (50)	
Age at onset of symptoms in years – median (range)	32.1 (1.4–62.2)	32.7 (16.4–55.0)	30.0 (1.4–50.2)	33.2 (10.1–62.2)	0.45
Age at diagnosis in years – median (range)	41.3 (1.4–72.2)	41.6 (14.6–61.0)	41.7 (1.4–63.8)	40.1 (23.8–72.2)	0.55
Serum creatine kinase (U/l)-median (range)	432 (96-3465)	351 (197–3465)	532 (96–1627)	471 (111–1481)	0.35
GAA activity in leucocytes* – median (range)	1.0 (0–6.5)	0.8 (0.0–5.0)	1.2 (0.0–6.5)	1.7 (0.0–2.9)	0.63
GAA activity in fibroblasts** – median (range)	12.9 (0.5–19.9)	12.7 (7.3–18.3)	13.0 (7.4–18.4)	13.0 (0.5–19.9)	0.88
Age at start of ERT in years – median (range)	52.9 (26.2–74.0)	56.9 (26.2–69.2)	48.7 (30.5–71.7)	52.5 (35.7–74.0)	0.60
Duration of disease in years – median (range)	8.2 (0.1–31.2)	8.8 (0.8–31.2)	9.1 (0.2–29.1)	4.8 (0.1–27.0)	0.34
MRC sumscore in percentage – median (range)	80.8 (48.3–93.9)	80.0 (56.9–91.5)	77.7 (53.3–93.9)	82.7 (48.3–93.1)	0.41
Wheelchair use – No. of patients (%)					
- No wheelchair use	49 (67)	20 (67)	17 (63)	12 (75)	0.64
- Partial wheelchair use	17 (23)	7 (23)	7 (26)	3 (19)	
- Permanent wheelchair use	7 (10)	3 (10)	3 (11)	1 (6)	
FVC uprigth in % predicted – median (range)	71.9 (11.3 – 125.4)	71.9 (20.8 – 125.4)	68.2 (16.4 – 115.7)	75.7 (11.3 – 105.8)	0.55
FVC supine in % predicted – median (range)	54.0 (23.0–119.6)	44.5 (25.0 – 119.6)	58.2 (23.0 – 109.6)	56.9 (23.9 – 89.3)	0.80
Mechanical ventilation – No. of patients (%)					
- No mechanical ventilation	51 (70)	18 (60)	21 (78)	12 (75)	0.26
- Non-invasive mechanical ventilation	18 (25)	8 (27)	6 (22)	4 (25)	
- Invasive mechanical ventilation	4 (5)	4 (13)	0 (0)	0 (0)	

* Acid α-glucosidase (GAA) activities in leucocytes are expressed as nmoles glucose/mg.hr and were measured in the presence of 3 μM acarbose (control range: 48-215 nmoles/mg.hr, n = 313)

** Activities in fibroblasts are expressed as nmoles 4MU/mg.hr (control range: 40-180 nmoles/mg.hr)

p-values for the exponential term were calculated in R-3.0.2. A p-value lower than 0.05 (two-sided) was considered statistically significant.

RESULTS

Patients

Seventy-three patients met the inclusion criteria as they had started ERT at least three years ago and had blood samples available for antibody testing just before start of ERT and during ERT at 6, 12 and approximately 36 months. At the last ELISA measurement, the median treatment duration was 35 months (range 24 – 41 months). Of the 73 patients, 72 received ERT during the entire study follow-up. In one patient ERT was discontinued after 33 months due to a poor response to ERT, the presence of IARs and co-occurrence of a very high antibody titer.[29]

The general characteristics of these patients at the start of ERT are shown in **Table I**. Male and female gender were evenly represented. Age of onset among the 73 patients differed widely between 1.4 and 62 years of age with a median age of 32 years. Enzyme replacement therapy was started at a median age of 52 (range 26 and 74 years) after a median disease duration of 8 years. One third of the patients was wheelchair dependent and 30% used mechanical ventilatory support. All but one carried the common c.-32-13T>G (IVS1) mutation on one allele in combination with a second pathogenic mutation on the second allele. The most common second mutations were c.525delT in 44% of cases and c.2481+102_2646+31del (delex18) in 15% of cases. For three patients the results of the mutation analysis could not be traced, because these were performed in another center.

Anti-rhGAA antibodies

Seventy-one patients (97%) developed antibodies to alglucosidase alfa. The highest titer for the whole group was reached after 6 months ERT, as the geometric mean titer was 1:1259 (95% CI 1:696 to 1:2,276). The geometric mean titer, thereafter, declined to 1:270 (95% CI 1:123 to 1:595) after 36 months of ERT (**Figure 1A**, bold line). Individual titers varied widely. Three groups could be defined according to the highest titer observed: 16 patients (22%) developed high titers (≥1:31,250), 27 patients (37%) intermediate titers (1:1250 – <1: 31,250), and 30 patients (41%) no to low titers (0 – <1:1250) (**Figure 1B**). These three groups showed similar mean trends compared to the total group (**Figure 1A**).
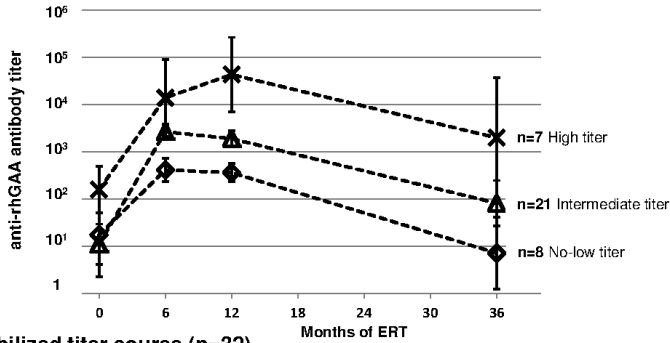
Figure 2 Different anti-rhGAA antibody titer courses stratified for the anti-rhGAA antibody titer groups during 36 months of enzyme replacement therapy
A – C Geometric mean and 95% confidence intervals of anti-rhGAA antibody titer course during enzyme replacement therapy for patients as stratified for the three anti-rhGAA antibody titer groups: **A** decreasing titer course; **B** stabilized titer course; and **C** increasing titer course (no 95% confidence intervals could be given due to small numbers). The titer course was defined as decreasing/increasing when the 36 months ERT titer was at least one dilution lower/higher compared to the 6 or 12 months titer.

However, within these groups, the course varied among patients. Three courses could be observed: a decreasing course, a stabilizing course, and an increasing course (**Figure 2**). The majority of patients (93%) showed either stabilizing or decreasing titers over time.

When demographic and clinical characteristics at start of ERT were compared across the three antibody titer groups many characteristics were similar (**Table I**). However, some of the clinical characteristics were different between the three groups, such as disease duration (shortest in the high titer group), partial or permanent wheelchair use (lowest prevalence in the high titer group), FVC_{supine} and serum creatine kinase (all lowest in the no-low titer group). All four invasively ventilated patients belonged to the no-low titer group. Although the observed differences did not reach statistical significance, when taken together, a trend was observed for a worse clinical status for the no-low titer group and a better clinical status for the high titer group. No differences in enzymatic activities or prevalence of a specific genotype were observed.

Effects of antibodies on alglucosidase alfa enzymatic activity

Antibodies to alglucosidase alfa have the potential to interfere with the enzymatic activity or uptake of alglucosidase alfa into cells. To test this, sera from patients were admixed with alglucosidase alfa in cell culture medium in which GAA-deficient fibroblasts were grown. After 24 hours, enzymatic activity was measured in both the medium and the cells. Reduction of activity in the medium is indicative of direct effects on enzymatic activity, while disproportional reduced activity in cells indicates reduced uptake. This was tested using blood samples from seven patients with high titers, three with intermediate titers, and three with no-to low titers. *In vitro* studies for the one patient with a very high titer of whom results were published previously were not repeated.[29]

Enzymatic activity was not inhibited by sera of patients with no-low to intermediate titers (**Figure 3A,B**). Blood samples of patients with high titers showed inhibition of enzymatic activity in both the medium and the cells, but the degree of inhibition in the medium and the cells varied per patient (**Figure 3A,B**). A very strong correlation (Spearman ρ=0.81, p=0.001) was observed between the activities as measured in the medium and in the cells, suggesting that the predominant and relevant effect of the antibodies was inhibition of enzymatic activity rather than other effects such as inhibition of uptake of alglucosidase alfa by the cells (**Figure 3C**).

Association between anti-rhGAA antibodies and infusion-associated reactions

Thirteen patients (18%) showed clinically confirmed IARs (**Table II**), of whom 12 were female (p=0.001). A strong relationship between IARs and the highest antibody titer was observed. First, the number of patients that experienced IARs was seven out of 16

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A

B

C
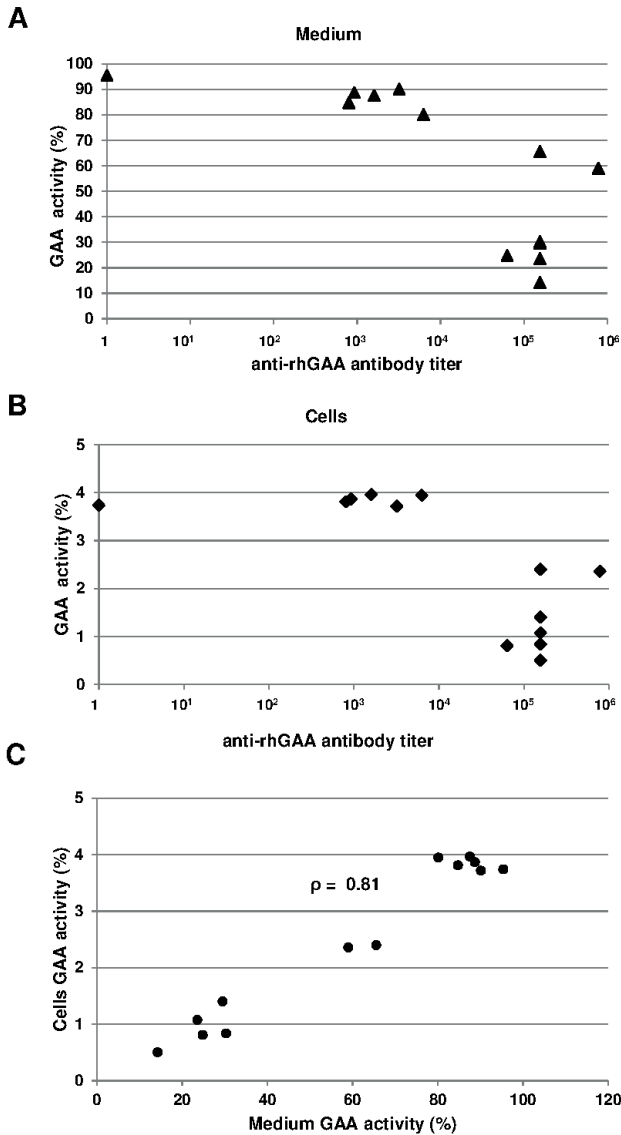
Figure 3 Effects of anti-rhGAA antibody titers on *in vitro* alglucosidase alfa enzymatic activity
Tests were performed for 13 patients, of whom 7 with a high titer, 3 with an intermediate titer, and 3 with a low or no titer. **A** and **B** show the alglucosidase alfa enzymatic activities in relation to the height of the antibody titer in the medium at the (**A**) and the cells (**B**; GAA-deficient fibroblasts) after 24 hours incubation. **C** Correlation between the rhGAA activities as measured in the medium and in the cells (Spearman ρ 0.81, p=0.001).

patients (44%) in the high titer group, five out of 27 (19%) in the intermediate titer group and only one out of 30 (3%) in the no-low titer group (p=0.001) (**Figure 4A**). Second, patients who had a high antibody titer experienced IARs more frequently (ρ 0.47, p<0.001; **Figure 4B**).

Most patients (nine) experienced their first IARs within the first year of treatment (median onset of IARs was 9 months of ERT, range 6 weeks to 35 months). Infusion-associated reactions usually commenced during the infusion of alglucosidase alfa or in the hours just after completion of the infusion. Only once, a patient suffered from an IAR the day after the infusion. Patients experienced various types of IARs (**Table II**), with general malaise, cold chills, and hyperthermia being the most frequently observed combination of symptoms (five patients). Almost all IARs were mild or moderate in nature, only one patient developed a severe IAR in the form of a transient hypertension up to 211/102 mmHg. To prevent further IARs, the infusion rates were slowed down in all but one patient. Additionally, pre-medication was given either in the form of antihistamines, corticosteroids, or as a combination of the two. At the end of the study IARs had resolved

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Table II Infusion-associated reactions

Type of IAR	Number of IARs No. (%)	Patients experiencing the IAR[a] No. (%)
General malaise	162 (22.7)	4 (31)
Fatigue	5 (0.7)	2 (15)
Cold chills	181 (25.3)	5 (38)
Itching	12 (1.7)	2 (15)
Flushing	2 (0.3)	2 (15)
Localized exanthema	8 (1.1)	4 (31)
Generalized exanthema	11 (1.5)	3 (23)
Local angioedema	1 (0.1)	1 (8)
Dyspnea	4 (0.6)	2 (15)
Chest discomfort	12 (1.7)	4 (31)
Musculoskeletal pain	74 (10.3)	2 (15)
Palpitations	3 (0.4)	1 (8)
Hypertension	20 (2.8)	8 (62)
Tachycardia	78 (10.9)	5 (38)
Hyperthermia	114 (15.9)	7 (54)
Desaturation	1 (0.1)	1 (8)
Headache	16 (2.2)	3 (23)
Dizziness	1 (0.1)	1 (8)
Nausea	9 (1.3)	3 (23)
Vomiting	1 (0.1)	1 (8)
Total	**715 (100)**	**13 (100)**
Total infusions with IARs	226	

[a] Patients may have had experienced more than one type of IAR.
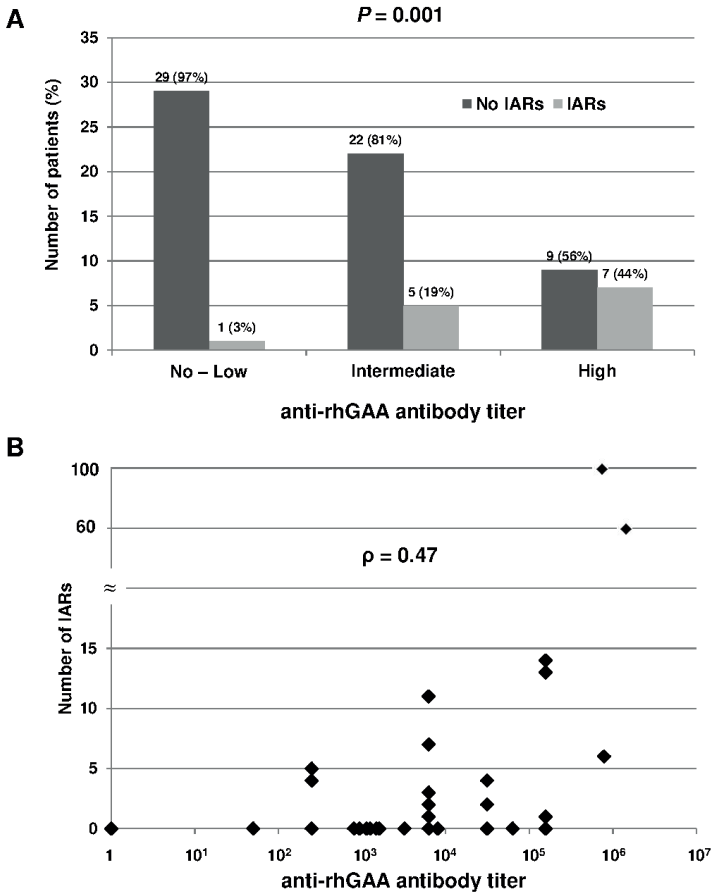
Figure 4 The association between infusion-associated reactions and the maximal anti-rhGAA antibody titer

A *shows the number of patients with and without infusion-associated reactions (IARs) for each anti-rhGAA antibody titer group.* **B** *Correlation between the number of IARs and the maximal anti-rhGAA antibody titer (Spearman ρ 0.47, p<0.001).*

in 11 of the 13 patients, two of whom continued to receive corticosteroids to prevent further IARs. All symptoms related to the infusions were reversible.

Association between anti-alglucosidase alfa antibodies and treatment outcome

To evaluate whether antibody formation interfered with clinical outcome, the pre-treatment course and the treatment course over the 36 months treatment period were

calculated for the three clinical outcome measures. Subsequently, the response to ERT was estimated by calculating the difference in course between the pre-treatment and the treatment period over 36 months of ERT.

For 58 patients pre-treatment data on disease progression were available with a median follow-up of 16 months (range 4 to 55 months). For all patients data were available during the treatment period. **Table III** gives the results in the total patient population and stratified for the three antibody titer subgroups.

During the pre-treatment period, the clinical outcome measures significantly declined at -0.9 pp/y for MRC sumscore, -1.7 pp/y for FVC in upright position, and -2.1 pp/y for FVC in supine position in the total patient population. The mean treatment course for MRC sumscore was 2.1 pp (p=0.001) during 36 months of ERT, whereas the treatment course of FVC in upright and supine position stabilized. The responses to ERT were found to be 5 to 6 pp for all three outcome measures (MRC sumscore p<0.001; FVC in upright and supine positions p<0.01).

With regard to the subgroup analysis, similar results across the three antibody titer groups were obtained for the treatment course of MRC sumscore and FVC in upright and supine positions. For MRC sumscore and FVC in upright position, also no significant differences between the three titer groups were found with regard to the response to treatment. A co-incidental finding was that the pre-treatment course of FVC in supine position differed across the antibody titers groups (borderline significant, p=0.06), with a more rapid decline in patients with high titers and intermediate titers as compared to the patients with no-low titers. This difference in pre-treatment course also explains the observed difference in the response to ERT for FVC in supine position (borderline significant, p=0.05), because the treatment course did not differ between the subgroups.

As we wondered whether the individual treatment courses of the seven patients with very high titers (≥1:156,250) showed an ongoing decline during ERT, we studied these courses separately (**Figure 5**). The patients with the highest titer of all patients (1:3,906,250) was the only patient with a rapid decline with regard to MRC sumscore during ERT. His FVC in both upright and supine position continued to decline after start of ERT, but less dramatically than his muscle strength (results published previously).[29] There was one other patient, who showed an ongoing decline with regard to pulmonary function, which was most pronounced for FVC in supine position. For the other patients with titers equal or higher than 1:156,250 muscle strength increased and FVC in upright and supine position remained fairly stable during ERT. Of note, among the patients with lower anti-rhGAA antibody titers there are also some patients, who continued to decline with regard to muscle strength and pulmonary function (data not shown), but not as rapid as the decline observed in the patient with the highest titer of all.

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Table III Natural course, treatment effect and response at 36 months enzyme replacement therapy for the total group and the antibody titer groups

	Pre-treatment course		Treatment course 36 months ERT		Response to ERT[b] 36 months ERT	
	mean pp/y[a]	p-value	mean pp	p-value	mean pp	p-value
MRC sumscore						
Total group (n=73)	-0.9	**<0.01**	2.1	**0.001**	4.8	**<0.001**
Subgroups anti-rhGAA antibody titer						
- No-low titer n=30	-0.8		2.8		5.1	
- Intermediate titer n=27	-1.3	0.67*	2.2	0.76*	6.1	0.67*
- High titer n=16	-0.5		0.6		2.2	
FVC in upright position						
Total group (n= 68)	-1.7	**<0.01**	1.2	0.24	6.2	**<0.01**
Subgroups anti-rhGAA antibody titer						
- No-low titer n=26	-0.9	0.34*	1.6	0.77*	4.4	0.54*
- Intermediate titer n=26	-2.1		1.1		7.4	
- High titer n=16	-2.1		0.8		7.2	
FVC in supine position						
Total group (n=60)	-2.1	**<0.001**	-0.8	0.43	5.4	**<0.01**
Subgroups anti-rhGAA antibody titer						
- No-low titer n=23	-1.1	*0.06**	-1.8	0.75*	1.5	**0.05**
- Intermediate titer n=22	-2.0		0.1		6.2	
- High titer n=15	-3.3		-0.8		9.0	

*[a] For the reason that duration of follow-up differed for each patient before start of ERT, the pre-treatment course is expressed in percentage points per year (pp/y). [b] The response to ERT is defined as the difference in course between the pre-treatment and the treatment period. * P-value for the difference between the three anti-rhGAA antibody subgroups, in case there was a linear relation a trend test was used to estimate the p-value.*

DISCUSSION

This study represents the first systematic study analyzing the relation between the formation of anti-rhGAA antibodies in response to ERT and clinical outcome, as well as the occurrence of IARs in adults with Pompe disease. It is the largest group of 73 patients, with the longest treatment follow-up (36 months), reported to date. The analyses show that the vast majority of adult patients develop antibodies to alglucosidase alfa, but that there are various immune response patterns. Although, after an initial rise in antibody titer, the titer stabilized or decreased with continued ERT in the majority of patients (95%). A strong association was found between the height of the antibody titer and the occurrence of IARs. No marked adverse effects on clinical outcome were found in the group of patients with high titers (≥1:31,250). An exception was the one patient with the

A MRC sumscore

B FVC in upright position

C FVC in supine position
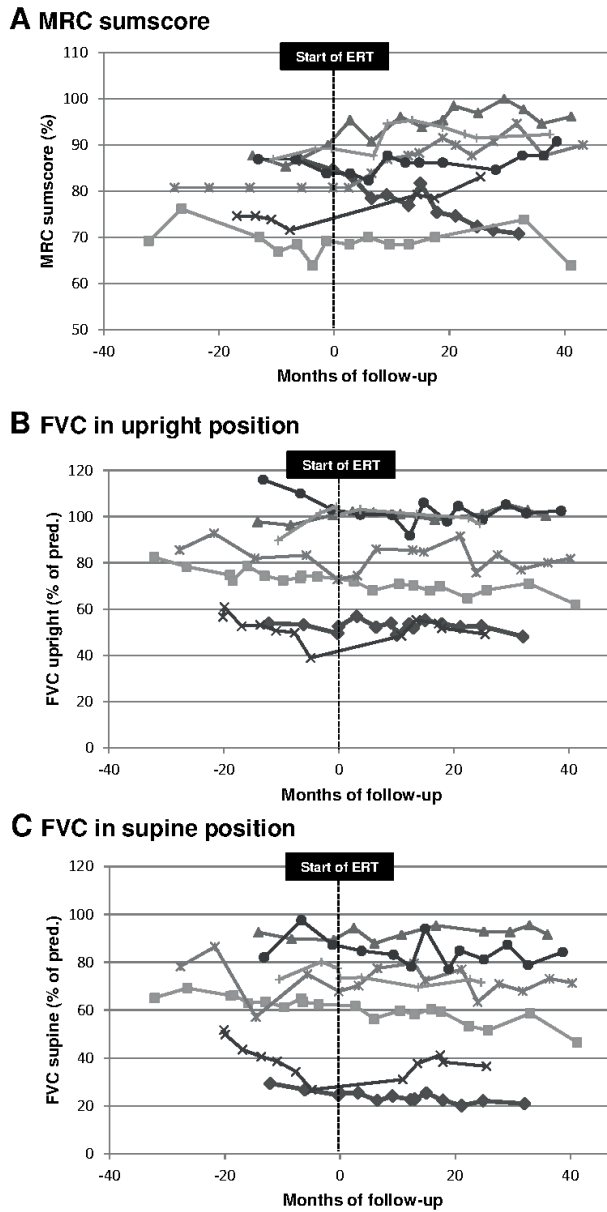
Figure 5 Individual courses of the MRC sumscore
(**A**) and FVC in upright (**B**) and supine positions (**C**) of patients with very high titers (≥1:156,250) before start of ERT and during ERT.

highest titer of all patients (1:3,906,250), who continued to decline rapidly with regard to muscle strength and moderately with regard to pulmonary function.[29]

Immune responses to recombinant human enzymes are a well-known phenomenon in lysosomal storage diseases. They are encountered in Pompe disease,[8, 23, 26, 27] Gaucher disease,[36] Fabry disease,[37-39] and Mucopolysaccharidosis type I, II and VI.[34, 40-43] The proportion of patients that develop antibodies varies from 2% to 100%, with the lowest proportions seen in Gaucher disease.

The biggest concern is that antibodies counteract the efficacy of ERT, but no adverse effects of antibody formation on therapeutic efficacy were found in Gaucher disease[36] nor in Mucopolysaccharidosis type I, II and VI.[34, 40-43] In Fabry disease, however, the lowering of globotriaosylceramide (GL-3) clearance stalled in some seropositive patients, although this had no adverse effect on treatment outcome.[39]

In this study, we found that 71 out of 73 patients with Pompe disease developed antibodies, which is similar to the findings of LOTS where all 59 treated patients seroconverted.[23] We found that patients with high titers showed inhibition of the alglucosidase alfa activity although to different extents. No significant effects on uptake of alglucosidase alfa were found. This suggests that inhibition of activity was the primary effect of the antibodies rather than inhibition of cellular uptake. In LOTS none of the 59 treated patients tested positive for inhibition of enzyme activity.[23] Two of these patients were also tested in the present study. For one of these patients, who had a high titer, we did found strong inhibition of enzymatic activity in both the medium and the cells. This discrepancy may be explained by the use of different assays. For example, the ratio alglucosidase alfa/serum and the time of incubation may play a role. The experiment performed here shows that similar titers can have different effects on *in vitro* enzyme inhibition, highlighting that our assay can detect antibody specificity and quantitative differences.

Although the *in vitro* studies demonstrated significant inhibition of GAA enzymatic activity, no clear adverse effect on clinical outcome in the high titer group (≥1:31,250) was observed. As mentioned before, the exception was the patient who developed the highest antibody titer.[29] Patel *et al.* also reported three patients who developed high sustained antibodies (range 1:102,400 to 1:819,200) coinciding with a poor clinical response to ERT.[30] It could be that these individual patients produce high levels of antibodies with specific binding affinities for the catalytic site and mannose-6-phosphate moieties resulting in impaired therapeutic efficacy of alglucosidase alfa.[44]

Our group recently published on the relation between high sustained antibodies and clinical outcome in classic-infantile Pompe disease. In this study it was concluded that ELISA titers of 1:6250 and lower probably have no clinical significant effect on clinical outcome, whereas titer of 1:31,250 and higher may counteract the efficacy of

alglucosidase alfa in a dose of 20 mg/kg every other week.[28] The results of our study and LOTS indicate that antibodies seldom impede the effects of ERT in adults with Pompe disease,[23] and when they do, the critical threshold of the ELISA titers seems to lay higher than the observed threshold in classic-infantile Pompe disease.[26, 28, 30] For the reason that antibody formation has little impact on clinical outcome in adults with Pompe disease, there must be other factors that determine the observed variance in clinical outcome.

Another concern of anti-rhGAA antibody formation is that it may alter drug safety. Indeed, we found that patients with higher antibody titers were more likely to develop IARs. For the majority of patients, first onset of IARs was within the first year of treatment. Fortunately, most IARs were tolerated fairly well and through slowing down the rate of infusion in combination with the use of pre-medication, these diminished over time with continued administration of alglucosidase alfa.

The LOTS study could not establish a consistent relation between the formation of antibodies and the occurrence of IARs. This difference in findings could be explained by the fact that LOTS reported all IARs and that in our study symptoms or signs being clinically judged to be related to the IARs were defined as IARs. Moreover, it could be explained by differences in study population (12 patients receiving ERT participated in both studies) and in follow-up duration. This may also explain the differences in prevalence of IARs, 18% in our study cohort versus 28% in the LOTS treatment group and 23% in the LOTS placebo group).[23] A remarkable finding was that all but one of the patients who developed IARs were female. This was independent of age or pre/postmenopausal status. Interestingly, it has been found that females tend to respond better to ERT as compared to males,[19, 23] but it is unlikely that this is related to antibody formation, as no gender differences were observed for either the level of the maximal antibody titer or presence of inhibitory antibodies in the study performed here.

The current clinical practice in the Netherlands is to receive in-hospital infusions during the first year of ERT and thereafter transfer to a home treatment environment. Earlier implementation of home treatment would be preferable, as this has a positive effect on quality of life and reduces health care costs.[45] The finding that patients with no or a low titer have a very low risk for IARs may lead to shortening of the period of in-hospital infusions for these patients.

Unexpectedly, we found a trend that less severely affected patients at start of ERT seem to develop higher antibody titers against ERT, as compared to patients who are more severely affected. Second, we found that a faster pre-treatment decline of pulmonary function in supine position was associated with the development of high anti-rhGAA antibody titers. An explanation for the latter may lie in the fact that they had a higher forced vital capacity to begin with, and therefore had more to lose as compared to the group of patients with no-low antibody titers who had overall more advanced

disease. The reason that disease status before start of ERT seems to be correlated with susceptibility to antibody formation to alglucosidase alfa might be that a milder disease state would allow a better and more reactive immune system. Although the immune system is known to function during the initial stages of muscle regeneration through the action of macrophages,[46] it is unknown if and how this translates to the propensity of antibody formation.

To conclude, antibody formation does not substantially attenuate the clinical response in adults with Pompe disease at group-level. But in patients who develop exceptional high antibody titers, treatment efficacy may be adversely affected. A new finding of this study was that the higher the antibody titer, the higher the risk for IARs. Therefore, close monitoring of IARs is indicated in patients with substantial antibody titers.

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CHAPTER 3.4

First experience with enzyme replacement therapy during pregnancy and lactation in Pompe disease

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ABSTRACT

Background Enzyme replacement therapy (ERT) with alglucosidase alfa was registered as a treatment for Pompe disease in 2006. It is as yet unknown whether ERT can be safely applied during pregnancy and lactation.

Case A primiparous 40-year-old woman diagnosed with Pompe disease continued receiving ERT during pregnancy and lactation. Before pregnancy, she had moderate limb-girdle weakness and used nocturnal ventilation. During pregnancy, her clinical condition remained fairly stable until the 25th gestational week. Thereafter she experienced more problems with mobility and respiration. Fetal growth was normal as monitored by regular ultrasound investigations. A healthy boy was born at a gestational age of 37 weeks and 5 days by elective Cesarean section. There were no maternal complications and the child developed normally. One year after delivery the mother's physical condition was similar as prior to her pregnancy. Pharmacokinetic studies following enzyme infusion showed that alglucosidase alfa was secreted into the breast milk. Activity levels in the milk (245 nmol/ml.h) peaked at 2.5 hours after the end of the infusion; which was 2 hours later than in the plasma (80 µmol/ml.h). Twenty-four hours after start of the infusion, the enzyme activity in the breast milk was back to the pre-infusion level.

Conclusions In this case report, the continuation of treatment with alglucosidase alfa during pregnancy and lactation has been safe for the mother and the child.

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INTRODUCTION

Pompe disease (OMIM 232300) is an autosomal recessive glycogen storage disorder caused by acid α-glucosidase deficiency due to mutations in the GAA gene. The clinical spectrum ranges from a very severe and rapidly progressive form in infancy to a more slowly progressive form later in life. Adults endure limb-girdle muscular weakness and respiratory insufficiency.[1]

In 2006 alglucosidase alfa (Myozyme®) was approved for enzyme replacement therapy (ERT) in Pompe disease but so far, no studies have been published about the continuation of ERT during pregnancy and lactation. In other lysosomal storage diseases the experience with continuation of ERT during pregnancy, in much lower dosages compared to Pompe disease, did not suggest major problems, and the product information of alglucosidase alfa mentions normal fetal development in mice and rabbits.[2-6] Based on this information we reasoned that continuation of ERT during pregnancy was not likely to pose a substantial risk to the mother or the fetus.

Here, we report about a 40-year-old woman, who continued to receive ERT during pregnancy under close monitoring. Additionally, we investigated the potential transfer of alglucosidase alfa into the breast milk.

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CASE

Case History
A primiparous 40-year-old woman was diagnosed with Pompe disease at the age of 22 on the basis of acid α-glucosidase deficiency caused by mutations in the GAA gene (c.-32-13T>G/c.1799G>A). Over the years, her limb-girdle weakness gradually progressed and she became dependent on nightly mechanical ventilation at the age of 36.

Before conception, her partner was tested for mutations in the GAA gene. None were found. Just before pregnancy, she had a waddling gait, had to use the banister when climbing stairs and had difficulties lifting objects. Her forced vital capacity (FVC) in upright position was 44% and in supine position 31% of the predicted normal value. When she became pregnant, she had been receiving ERT for 17 months. She participated in a prospective observational study on the effects of ERT in Pompe disease, for which she provided written informed consent.

Pregnancy and Delivery
During pregnancy she was treated with 20 mg/kg alglucosidase alfa every other week. Her clinical condition remained fairly stable during the first two trimesters (**Figure 1**).
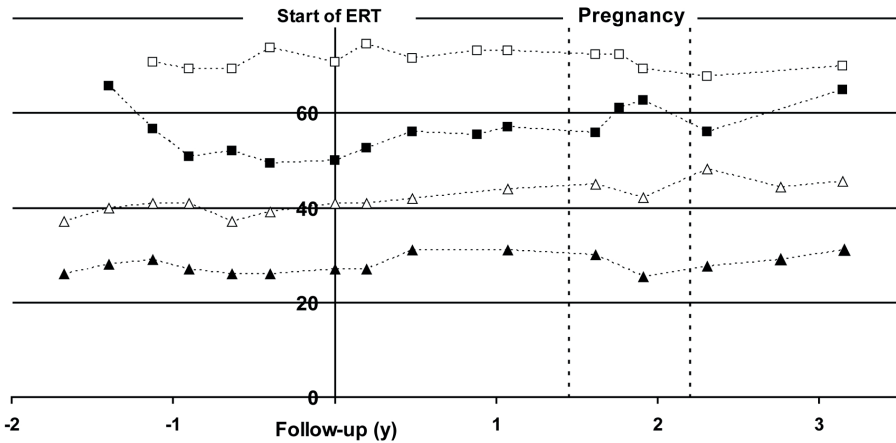
Figure 1 Muscle strength and pulmonary function before and after start of enzyme replacement therapy, including the pregnancy period
Muscle strength was measured with manual muscle testing (□) (the MRC sum score of 26 muscle groups is expressed as percentage of normal muscle strength). The muscle strength was also measured with hand-held dynamometry (■) (the sum score of 16 muscle groups is expressed as percentage of the median strength for healthy women). Pulmonary function; the forced vital capacity (FVC) is expressed as percentage of the predicted value in upright (Δ) and supine (▲) position.

From the 25th gestational week onwards she experienced more mobility and respiratory problems. Nearing the end of her pregnancy, the FVC in upright position decreased to 42% and the FVC in supine position to 25% (**Figure 1**). Normoventilation was maintained by increasing the ventilatory frequency and pressure during night-time ventilation. The fetal growth appeared normal as monitored by regular ultrasound investigations.

Due to the increasing dyspnea, an elective Cesarean section was performed at a gestational age of 37 weeks and 5 days. Combined spinal-epidural anesthesia was ap-plied,[7] while the patient used her own non-invasive ventilation. There were no maternal complications. A small strip of myometrium was dissected, which showed lysosomal glycogen storage in the smooth muscle cells and in the tunica media of blood vessels. There was no gross tissue damage (**Figure 2**).

A healthy boy was born (Apgar scores of 8, 9 and 10) with normal birth weight, length and head circumference, and no congenital anomalies. The pH of the cord blood was 7.29. Regular physical examinations of the child up to 1 year of age revealed a normal development.

In the early postpartum period the mother suffered from overstrain, which was re-flected in a temporarily decrease in muscle strength. Her dyspnea diminished fairly soon
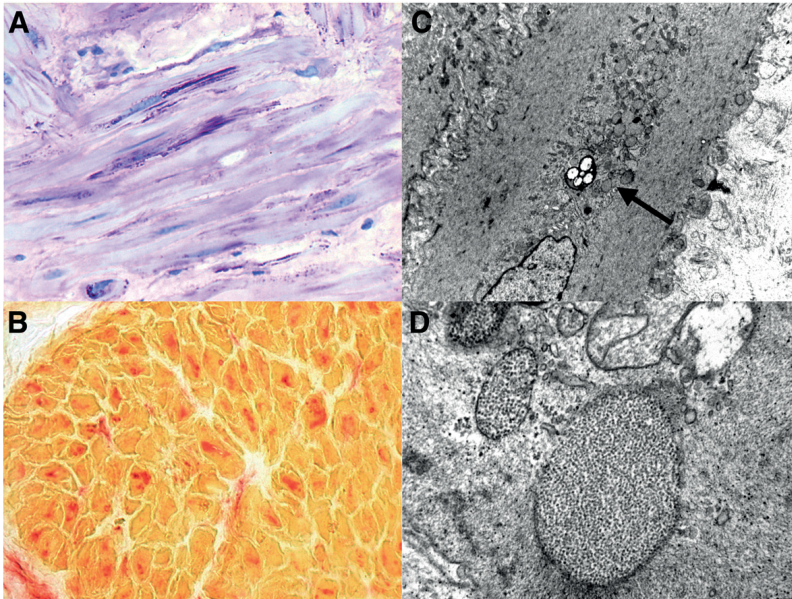
Figure 2 A) Periodic acid Schiff *(PAS)* staining of a section of the myometrium demonstrates glycogen accumulation in smooth muscle cells; **B)** A section of the myometrium stained for acid phosphatase (red color) demonstrates lysosomal pathology; **C** and **D)** Electron microscopic images demonstrate lysosomal glycogen accumulation in a smooth muscle cell of the myometrium. The area indicated with an arrow in **C** is magnified in **D** showing a single lysosome filled with glycogen particles.

after the delivery, and one year postpartum her pulmonary function was even slightly better than before the pregnancy.

Lactation

Before, during and after the first postpartum infusion, the activity of acid α-glucosidase was measured in the plasma (500 fold diluted) and in the breast milk (undiluted) (**Figure 3A**).[8] Before infusion, the activity in the milk was 3 nmol/hr.ml, which is approximately 10% of what we measured in the milk of an unaffected mother. During infusion the activities in the milk and the plasma increased. The highest activity in the milk (245 nmol/hr.ml) was reached at 2.5 hours post-infusion, 2 hours later than in the plasma (80 μmol/hr.ml), and was only 0.3% of the peak plasma value.

The presence of endogenous acid α-glucosidase in the milk of an unaffected mother could be demonstrated by SDS-PAGE immunoblot analysis, but the same method detected hardly any enzyme in the pre-infusion milk of the patient (**Figure 3B**). The milk collected after enzyme infusion contained a substantial amount of alglucosidase alfa as demonstrated by the strong fluorescent signal.

A

B
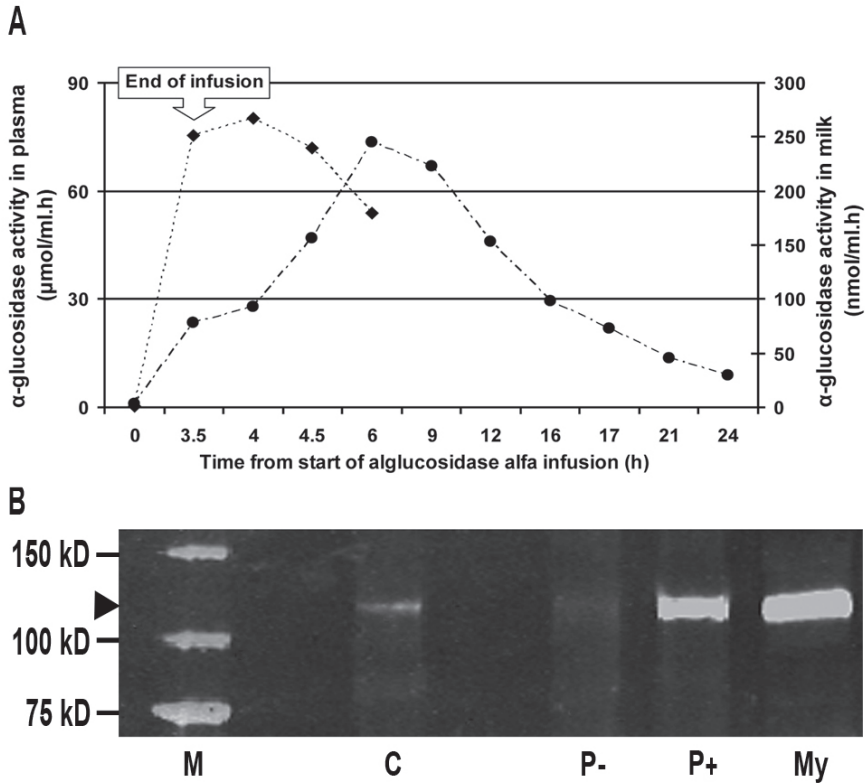
Figure 3 A) Pharmacokinetic analysis of alglucosidase alfa activity in plasma and breast milk during and after infusion. The administration of alglucosidase alfa was stepwise increased according to the regular infusion schedule.(7) Blood samples (◆) and milk samples (●) were collected just prior to the start of the infusion (0 h) and at regular time intervals thereafter as indicated in the figure. The acid α-glucosidase activity in the plasma is expressed in μmole/ml.h and in breast milk in νmol/ml.h. **B)** SDS-PAGE immunoblot analysis to demonstrate the presence of endogenous acid α-glucosidase and alglucosidase alfa in breast milk. Alglucosidase alfa has the same molecular mass (110 kD) as the endogenously produced precursor form of acid α-glucosidase (arrow head). M = molecular marker; C = milk sample from an unaffected mother; P- = pre-infusion milk sample from the affected mother; P+ = post-infusion milk sample from the affected mother; My = alglucosidase alfa (Myozyme®).

Anti-alglucosidase alfa antibody titers

At day 3 and day 77 after delivery, the mother and child tested negative for anti-alglucosidase alfa antibodies using the immuno-precipitation method as previously described.[8]

The antibody titer was also determined by using an Enzyme-Linked ImmunoSorbent Assay (ELISA). To this end, F96-Maxisorp Nunc-Immuno plates were coated with a 50 μl solutions of 5 μg/ml alglucosidase alfa in phosphate buffered saline (PBS). After blocking

with a 250 µl solution of 1% bovine serum albumin in PBS, the serum of the mother and the child were added to the wells in two-fold serial dilutions (50 fold up to 3200 fold), and the plates were incubated for 1 hr at room temperature. The plates were then washed 6 times with PBS containing 0.05% Tween-20, and subsequently incubated for 1 hr with 50 µl of an HRP-conjugate (Acris Antibodies Shop, R1343-HRP). After a second wash, the colour (450 nm) was developed by the addition of 100 µl 3,3',5,5' – tetramethylbenzidine (TMB) for 10 min, and 100 µl of 1M ortho-phosphoric acid to stop the reaction. Sera from unaffected individuals were used as negative controls and a mouse serum that was raised against alglucosidase alfa was used as a positive control. Readings above two times the negative control were taken as titer.

At day 3 post-partum, this method revealed very low titers of 1:800 in the mother and 1:400 in the baby.

DISCUSSION

This case report indicates that alglucosidase alfa can be safely administered during pregnancy in Pompe disease; the condition of the affected mother remained fairly stable and both the prenatal as well as the postnatal development of her child were uneventful.

Continuation of ERT during pregnancy has become common practice in Gaucher disease and in Fabry disease.[2-6] Nevertheless, we were cautious with our decision to continue ERT in Pompe disease since the prescribed dose of alglucosidase alfa is up to 20 fold higher than the dose of any other recombinant human enzyme administered to patients with lysosomal storage disorders. The mother is likely to benefit from continued treatment, but the fetus might be exposed to alglucosidase alfa or any of its constituents that might cross the placental barrier. From animal studies, it is known that cross-placental transfer of alglucosidase alfa hardly occurs, but IgG can cross the placental barrier.[2] Therefore we measured the occurrence of anti-alglucosidase alfa antibodies in the serum of the mother and the child shortly after birth. We did not measure any substantial immune response as compared to the high antibody titers that may occur in patients receiving ERT.[8,9] Evidently, one of the best proofs of safe practice is the uneventful course of the pregnancy and the normal development of the fetus through all gestational stages.

As opposed to potentially negative effects that continuation of ERT during pregnancy might have on the fetus, it might have positive effects on the mother regarding muscle strength and pulmonary function.[9] The latter is excessively compromised during pregnancy due to the expanding uterus. The pulmonary function of our patient decreased slightly during pregnancy, but there was fortunately no need for invasive ventilation. Continuation of ERT during pregnancy might also counteract the lysosomal glycogen

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storage in the myometrium that could affect the uterine strength and contractility needed for natural childbirth. To minimize the risk for mother and child an elective Cesarean section was performed using combined spinal-epidural anaesthesia.[7]

We found low levels of alglucosidase alfa in breast milk at the time of infusion. Interestingly, the activity in the milk peaked 2 hours later than in the plasma, which roughly indicates how long it takes for alglucosidase alfa to cross the endothelial barrier, diffuse through the interstitium and pass the epithelial lining of the mammary gland. Within 24 hours after infusion, the activity in the milk was back to the pre-infusion level. Our findings parallel the recently reported secretion of imiglucerase in the breast milk of a woman receiving ERT for Gaucher disease.[10]

There seems little rationale to entirely refrain from breastfeeding while mothers are on ERT, but to be on the safe side we have advised to discontinue breastfeeding from the start of infusion until 24 hours later.

We conclude that the continuation of enzyme replacement therapy during pregnancy and lactation has been safe for the mother and the child described in this case report. But, we underline that our experience is limited to this single case and that careful monitoring of each pregnancy is required.

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Chapter

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4.1

CHAPTER 4.1

Fatigue in neuromuscular disorders: focus on Guillain–Barré syndrome and Pompe disease

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ABSTRACT

Fatigue accounts for an important part of the burden experienced by patients with neuromuscular disorders. Substantial high prevalence rates of fatigue are reported in a wide range of neuromuscular disorders, such as Guillain–Barré syndrome and Pompe disease. Fatigue can be subdivided into experienced fatigue and physiological fatigue. Physiological fatigue in turn can be of central or peripheral origin. Peripheral fatigue is an important contributor to fatigue in neuromuscular disorders, but in reaction to neuromuscular disease fatigue of central origin can be an important protective mechanism to restrict further damage. In most cases, severity of fatigue seems to be related with disease severity, possibly with the exception of fatigue occurring in a monophasic disorder like Guillain–Barré syndrome. Treatment of fatigue in neuromuscular disease starts with symptomatic treatment of the underlying disease. When symptoms of fatigue persist, non-pharmacological interventions, such as exercise and cognitive behavioral therapy, can be initiated.

Chapter
4.1

INTRODUCTION

Everybody experiences periods of fatigue, but these usually can be mended by taking a rest or enjoying a good night's sleep. In a wide range of diseases, however, fatigue is a well-known chronic symptom. In patients without demonstrable somatic disease chronic fatigue may occur and may lead to the diagnosis of chronic fatigue syndrome.[31] Severe fatigue as a chronic symptom and its impact on daily living are starting to receive more and more attention as patient-reported outcome measures in clinical trials in patients with somatic disease. Several studies have demonstrated that fatigue is a disabling symptom in chronic diseases such as cancer, but also in a variety of neurological disorders and conditions (**Table I**).[14] Severe fatigue can also occur as a side effect of drugs like beta-blockers, proton-pump inhibitors, anxiolytic drugs and antipsychotics.[2]

The first studies on fatigue in neurological diseases focused on diseases of the central nervous system (CNS), such as cerebrovascular disease, multiple sclerosis and Parkinson's disease.[29, 30, 44, 54, 88, 106] Nowadays, it is recognized that fatigue is also an important and frequently occurring symptom in disorders of the peripheral nervous system (PNS) with a high impact on physical abilities and perceived health status in these patients.[104]

This review focuses on fatigue in neuromuscular disorders, with emphasis on fatigue in Guillain–Barré syndrome (GBS), an immune-mediated polyradiculoneuropathy, and Pompe disease, a metabolic muscle disorder. Various aspects of fatigue will be discussed, including differences between experienced and physiological fatigue and different methods available for assessment of fatigue. GBS and Pompe disease are taken as examples to discuss possible pathological mechanisms of fatigue, treatment strategies and options for future research.

Chapter

4.1

DIFFERENT TYPES OF FATIGUE AND THEIR DEFINITIONS

Fatigue covers a broad spectrum of symptoms and complaints, and has no uniform definition. In clinical research, commonly used definitions of fatigue are an overwhelming and persistent feeling of tiredness and diminished ability to sustain voluntary mental and physical activities. In addition, weakness, lethargy and lack of energy that interfere with daily activities are used as definitions.[9, 13, 22, 23, 29, 51, 53, 57, 107] In basic neurosciences, it is defined as a time-related force decline.[32,102] Considering the different aspects of fatigue it is best regarded as a multidimensional concept in which the level of experienced fatigue and the ability to perform activities are influenced by the type of disease, the health status of the patient and several aspects of patient functioning. A distinction is made between experienced fatigue and physiological fatigue, which can both be influenced

Table I Neurological disease or condition associated with fatigue

Cerebral commotion
Cerebral vasculitis and cerebrovascular diseases
Channelopathies
Charcot-Marie-Tooth disease type 1 (CMT type 1)
Chronic inflammatory demyelinating polyneuropathy (CIDP)
Developmental disorders (cerebral palsy, Arnold-Chiari malformations)
Dysautonomic states
Encephalitis
Facioscapulohumural dystrophy (FSHD)
Granulomatous disorders (neurosarcoidosis, Wegener's granulomatosis)
Guillain-Barré syndrome (GBS)
Hypothalamic and pituitary diseases
Intracranial infections (meningitis and encephalitis)
Metabolic encephalopathy and mitochondrial diseases
Migraine
Motor neuron disease
Multiple sclerosis
Multiple system atrophy
Myotonic dystrophy
Narcolepsy and related sleep disorders
Paraneoplastic (limbic encephalitis, opsoclonus-myoclonus)
Parkinson's disease and other parkinsonian disorders
Pompe disease and other metabolic myopathies
Post-infection fatigue states (poliomyelitis, Lyme disease, Q-fever, and viral infections)
Postoperative (posterior fossa) surgery

Table adapted from Chaudhuri et al., The Lancet, 2004.[14]

by psycho-sociological factors.[101] The multidimensional concept of fatigue is depicted in **Figure 1**. In this figure fatigue is integrated in the World Health Organization's International Classification of Functioning, Disability and Health (WHO-ICF). The WHO-ICF represents the effect of disease on body function and structure and the level of activities and participation of the patient.[1]

Physiology of fatigue

With electrophysiological testing, fatigue can be measured as a loss of voluntary force-producing capacity of the muscles during exercise.[8,72] To understand this reduced capacity of the muscle it is important to know how fatigue arises. The central governor model clarifies in a logical way how fatigue arises during mental or physical effort and how fatigue is essential for protecting the body against damage due to excessive exercise.[69] During performed effort the CNS is continuously informed about the level of perceived
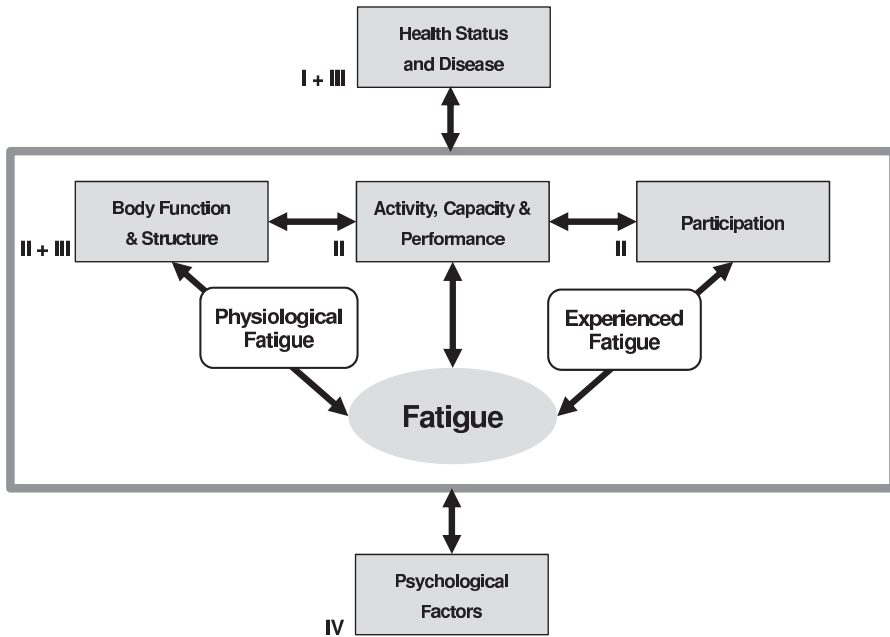
Figure 1 Fatigue as a multidimensional concept implemented in the World Health Organization's Classification of Functioning, Disability, and Health
The multidimensional concept of fatigue is integrated in the World Health Organization's International Classification of Functioning, Disability and Health (WHO-ICF), representing the effect of disease on body function and structure, activity and participation of the patient.[1] Both experienced fatigue and physiological fatigue have an effect on activity and participation and are in most diseases related to health status and disease severity. Psychosocial factors have an influence on fatigue and on activity and participation. The numbers indicate at which level the different treatment strategies have an effect on fatigue: I treatment of underlying disease; II rehabilitation and exercise; III pharmacotherapeutics; IV cognitive behavior therapy.

exertion by feedback from muscles, joints, body temperature, the cardiorespiratory system and cognitive domains (afferent pathways). This feedback is processed at the primary somatic sensory cortex, subsequently sensory motor integration takes place and the activation by the primary motor cortex of the brainstem motor nuclei and anterior horn cells in the spinal cord (efferent pathway) is adjusted if needed to maintain body homeostasis. This continuous feed forward and feedback control mechanisms regulate the work rate and determines when rest is needed.[14,69]

With this model it is not fully explained how severe fatigue can be present in chronic disease or how fatigue can be present in a resting situation. In patients with severe fatigue the sense of normal fatigue is amplified due to pathological changes in the motor system, disruption of feedback to the primary somatic sensory cortex and/or change in

patients' motivation.[14,69] In a resting situation an estimation is made whether the body can cope with an increase in effort. Up-regulation of metabolism occurs during exercise; however, in case of disease, the capacity to increase the metabolic rate is limited. This is perceived at the level of the CNS as fatigue and the patient will avoid excessive effort and requires rest.

In systemic diseases loss of voluntary force-producing capacity is caused by multi-systemic consequences of the underlying disease. In neurological disease a distinction can be made between fatigue caused by disease at the level of the CNS (central fatigue) versus fatigue caused by disease at the level of the PNS (peripheral fatigue). Neurological diseases can be located at various levels of the nervous system, such as the motor cortex, the spinal cord, the peripheral nerve, the neuromuscular junction or the muscle itself.[107] Most of these structures are either important feedback pathways for the perceived effort or important in voluntary force-producing capacity of the muscle. Presumably for that reason, severe fatigue is commonly seen in a variety of neurological diseases.

Central fatigue
Central fatigue is the term generally used when symptoms result from either psychiatric disease or pathology in the CNS. This can result in either difficulty with sustaining physical activities and/or impaired cognitive functioning. For example, patients with structural lesions in brain areas important for cognitive processing will have limited ability to sustain concentration and endure mental tasks (mental fatigue).[5, 11, 13, 14] Psychiatric diseases may disturb neuronal processing in the CNS, for example, in depression there is reduced internal input from the CNS, which results in loss of interest and motivation and an exaggerated sense of fatigue.[14] Loss of voluntary force-producing capacity of the muscles during exercise due to central mechanisms seems to result from suboptimal input from the CNS. This is observed in patients with diseases disrupting the motor cortex or the motor pathways of the CNS such as multiple sclerosis.[84] Endocrine disturbances as seen in hypothalamic-pituitary diseases may give rise to profound chronic fatigue.[14] Temporal hypothalamic-pituitary dysregulation is also responsible for fatigue as seen in the over-trained athlete syndrome.[3]

Peripheral fatigue
Peripheral fatigue, which is similar to muscle fatigability, is likely to occur in a patient showing a topographic pattern of muscle weakness or sensory disturbances at neurological examination based on dysfunction of the lower motor unit or sensory nerves. Patients with metabolic myopathies (e.g., Pompe disease), with disorders at the level of neuromuscular transmission (e.g., myasthenia gravis), with disorders affecting the peripheral nerves (e.g., GBS and Charcot Marie Tooth type 1 (CMT type 1)) and the anterior

horn cells (e.g., motor neuron disease) can all suffer from peripheral muscle fatigability, which is characterized by failure to sustain the force of muscle contraction over time. In patients with myopathies, muscle fatigability is enhanced due to structural changes or altered muscle metabolism in the muscle, which hampers muscle contraction. When motor conduction is impaired, this leads to decreased recruitment of motor units, resulting in an earlier occurrence of muscle fatigability.[14] Myasthenia gravis is characterized by rapid progressive reduction of muscle strength with repeated muscle contraction with (partial) recovery after a period of rest. This phenomenon of inability to sustain physical activities is caused by anti-acetylcholine (Ach) receptor antibodies and a reduction in the number of Ach receptors of the neuromuscular junction.[19]

Fatigue of central origin in neuromuscular disease

Interesting is the finding that patients with PNS disease also suffer from a generalized form of fatigue, which originates at the level of the CNS. This should not be confused with muscle weakness or (exercise induced) muscle fatigability. Previously, it was thought that generalized fatigue was caused by skeletal muscle hypoxia or anaerobiosis; however, these metabolic changes should lead to muscle rigor.[69, 73] Furthermore, one would expect that the primary motor cortex tries to compensate for the decrease of generated force by recruiting the maximum number of motor units; however, this is not seen during maximal exercise.[87] For example, in muscle disease, if muscle metabolism is beforehand affected, this will result in a continuous afferent feedback to the CNS. The CNS will minimize activities to protect further damage to the muscles (central governor model). In case of muscle weakness an increase in applied effort is needed to perform the same activities as a healthy person. This will lead to a higher perceived exertion at the level of the primary somatic sensory cortex and an earlier occurrence of fatigue.[14, 69]

In neuro-inflammatory diseases, e.g., post-poliomyelitis syndrome or chronic inflammatory demyelinating polyneuropathy (CIDP), fatigue may arise from dysregulation of the neuro-endocrine system. The local inflammatory process in the muscle or nerves may trigger a systemic inflammatory response, which causes neuro-endocrine dysregulation.

Experienced fatigue

Experienced fatigue is the patient's perception of his or her level of fatigue in daily life, which is alike to feeling or sense of fatigue.[14, 107] This reflects to what extent individual patients notice the increase in effort needed to complete daily activities and their limitations in endurance during sustained physical and mental activities.[107] It is a subjective form of fatigue. The way how fatigue is experienced is influenced by psycho-sociological factors of the patient, such as attributions towards fatigue, coping mechanisms, overall well-being and social circumstances (**Figure 1**).[101] For example, patients who are stress-

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sensitive or encounter a stressful event in life are often in a fight or flight state, characterized by activation of the sympathetic nervous system and initiation of the acute stress response via the hypothalamic–pituitary–adrenal axis. These prolonged stress responses may result in exhaustion and feelings of fatigue. Certain individual patient characteristics, such as emotional state and lack of motivation, may cause a dissociation between the level of internal input (motivational and limbic) and that of the perceived signal of performed effort.[14] This dissociation is responsible for an increased perception of fatigue. Experienced fatigue and physiological fatigue can be related to each other, but do not necessarily co-occur within individuals. In CMT type 1, facioscapulohumeral dystrophy (FSHD) and myotonic dystrophy, central fatigue (not peripheral fatigue) correlates with experienced fatigue; however, in GBS no correlation has been found between either peripheral or central fatigue.[35, 81]

HOW TO ASSESS FATIGUE

The broad range of mechanisms underlying fatigue, its multi-dimensional character, confounding factors and different manifestations of fatigue put high demands on the way fatigue is measured. It is often difficult to assess the difference in severity of fatigue among individuals. Nevertheless, quantification of fatigue and its impact on the patient's life are important for good insight in the overall condition of the patient and may be helpful in deciding which supportive measures should be taken to alleviate fatigue.[72]

Several methods have been developed to assess the level of fatigue. These measurements can be divided into two categories. The first one, generally based on patient-reported outcome measures, focuses on experienced fatigue. The second category is based on objective tests and outcomes, and focuses on physiological fatigue.[22, 58] The different techniques quantifying the severity of each form of fatigue will be discussed in the next paragraphs.

Ways to measure experienced fatigue

Experienced fatigue needs to be quantified via patient-reported outcome measures (questionnaires). Most of these questionnaires are designed for use in daily practice, can be completed in a limited amount of time and provide the physician with an estimate of the severity of fatigue and its impact on the patients' daily life. To measure treatment effects or fluctuations in severity of the underlying disease, a scale should be responsive to changes over time.[22] A distinction is made between unidimensional and multidimensional scales. A unidimensional scale approaches fatigue as a single construct, while multidimensional scales assess several aspects of fatigue, such as physical, cognitive and

psychosocial aspects. An elaborate review by Dittner et al.[22] gives a systematic overview of 10 unidimensional and 20 multidimensional scales for the measurement of fatigue. It also provides guidance on choosing the appropriate scale for a particular patient population.

An example of a unidimensional fatigue scale frequently used in neuromuscular disorders is the Fatigue Severity Scale (FSS). This test is easy to use in clinical practice, as the nine items of the test can be answered within less than 5 min. The FSS measures fatigue by assessing the consequences of fatigue on daily functioning. The scores of the scale can range from 1 (no fatigue) to 7 (extremely fatigued). An average score of 4 and higher is indicative for fatigue, and a score of 5 and higher for severe fatigue.[27, 40, 45, 56, 64] The FSS demonstrated good internal consistency (Cronbach's alpha coefficient=0.88), test–retest reliability (Cohen's kappa value=0.84) and discriminative validity in studies among patients with immune-mediated polyneuropathies, multiple sclerosis and Pompe disease.[22, 38, 40, 56]

An example of a multidimensional fatigue scale is the Checklist Individual Strength (CIS). This is a 20-item questionnaire that evaluates fatigue severity in general (i.e., not related to specific daily activities), concentration, motivation and the level of physical activity.[81] The CIS was not specifically validated for patients with neuromuscular disorders, but has shown good psychometric properties in other populations (internal consistency; Cronbach's alpha coefficient=0.90) and discriminative validity between multiple sclerosis and healthy controls).[7, 89, 97] The CIS has been used to assess fatigue in patients with chronic fatigue syndrome, multiple sclerosis, FSHD, CMT type 1 and myotonic dystrophy.[81, 83, 89, 97, 101]

Ways to measure physiological fatigue

There is limited experience with methods that have the potential to objectively quantify the level of physiological fatigue. The following methods can be used: electrophysiological measurement of decline in muscle force after exercise (peripheral fatigue); electrophysiological measurement of central activation failure (central fatigue)[107] and; neuropsychological assessment (central fatigue).[14]

The most direct method for measuring peripheral fatigue is measuring the generated force by electrical stimulation during rest and after muscle contraction and to look at the decline in generated force (**Figure 2**). The decline in force reflects the severity of fatigue. It also shows the change in muscle contractibility, such as a fatigue-induced delay of the muscle relaxation phase (**Figure 2**).[18] During the contraction a decline over time of the maximal voluntary force can be observed (muscle fatigability). The actual force exerted is usually expressed as a percentage of the maximal force (e.g., 70% of the maximal

voluntary contraction). With a surface electromyogram a decline in fiber conduction velocity may be indicative for peripheral fatigue.[107]

When central fatigue is present, suboptimal input from the CNS to activate the muscle results in a decrease in maximal force generating capacity of the muscle. This is defined as central activation failure (CAF). When during maximal voluntary contraction the generated force increases with electrical stimulation, this is indicative for CAF (**Figure 2**). When CAF increases during exercise, this is supportive for the presence of central fatigue. CAF can be confirmed with a twitch-interpolation technique, magnetic and electrical stimulation of the motor cortex and readiness potential. For a commentary on these methods, the review by Zwarts et al. is recommended.[107]

A method to evaluate mental fatigue is through neuropsychological assessment. Via this assessment cognitive domains of memory, learning, attention and information processing are evaluated. Patients with mental fatigue perform worse on these tests, and their performance will decline during the assessment session.[5, 11]
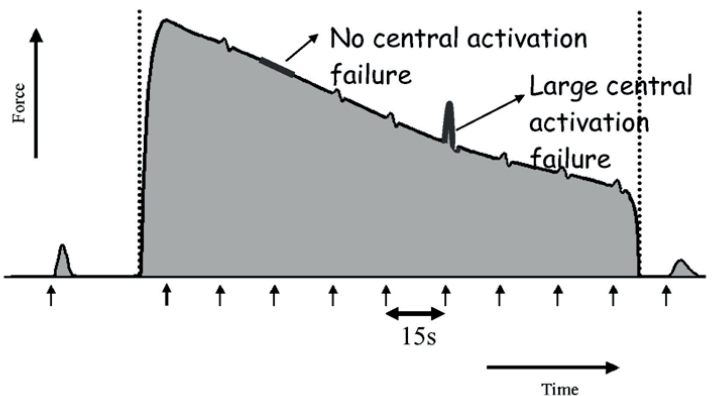
Figure 2 Schematic representation of physiological fatigue
The figure shows the decline over time of the maximal voluntary force (on the Y-axis). The 'at-rest twitches' are visible before and after the contraction, with the post-contraction twitch being clearly lower and slower, indicative of peripheral fatigue. The arrows indicate the moments of superimposed electrical endplate stimulation. The twitch interpolation has induced small increments in muscle force with examples of a negligible and a large central activation failure (CAF). A (near) absent response indicates a full voluntary activation of the muscle. This figure originates from Zwarts, et al., Clininical Neurophysiology 2008.[107]

FATIGUE IN NEUROMUSCULAR DISORDERS

Neuromuscular disorders include a wide range of disease entities causing abnormalities in muscle function and/or sensory function. These may be caused by dysfunction at the level of the muscle itself, the neuromuscular junction, the peripheral nerve or at the level of the anterior horn cell in the spinal cord or brain stem. Typical features of neuromuscular disorders comprise muscular atrophy, muscle weakness and sensory disturbances such as pain, tingling-feelings, hyperpathy or hypoesthesia.[19]

Fatigue has been an under-recognized aspect of neuromuscular disorders over a long period of time. As we have mentioned before, fatigue on one hand is caused by a local effect on muscle function (peripheral fatigue), and on the other hand fatigue originates at the level of the CNS because of feedback about the pathological state of the PNS. This fatigue protects the muscles from further damage by down-regulating physical activities. This is illustrated by the fact that fatigue of central origin was recorded in 36–41% of patients with a neuromuscular disorder (FSHD, CMT type 1 and myotonic dystrophy) vs. 12% in controls.[82] These high percentages, however, were not found in a small study in GBS patients.[35] In patients with FSHD, CMT type 1 and myotonic dystrophy, fatigue of central origin correlated positively with the level of experienced fatigue. In GBS patients, this correlation has not been found.[35, 81] It should be noted that most individuals with severe fatigue, but without demonstrable muscle weakness and/or elevated serum creatine kinase (CK) levels do not have a neuromuscular disorder. Thus, fatigue as the only complaint excludes in most cases the presence of a neuromuscular disorder.[59] An exception to this rule are mitochondrial myopathies or other metabolic disorders in which clear exercise intolerance can reported by patients where muscle weakness is not always present at the time of the neurological examination.[14] Furthermore, fatigue can persist as a residual symptom after a neurological illness, of which GBS and CIDP are good examples.[38, 64]

Other disorders in which increased levels of fatigue have been reported are myasthenia gravis, post-poliomyelitis syndrome, immune-mediated neuropathies, FSHD, CMT type I, myotonic dystrophy and Pompe disease.[38, 40, 43, 48, 53, 64, 71, 78, 81] The prevalence of fatigue in various neuromuscular disorders reported in the literature ranges from 38 to 86% (**Table II**). The reported prevalence of fatigue in neuromuscular disorders compares with the prevalences of fatigue in multiple sclerosis (range from 53 to 92%),[10] where fatigue is recognized as an important symptom.[54] To be aware of the impact of fatigue in neuromuscular disease, it is valuable to compare its high prevalence with the low prevalence of severe fatigue in healthy individuals (range 5–18% and mean FSS 2.3–3.3).[64, 78, 92] The ranges seen in the mentioned prevalences are explained by differences in study

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Table II Prevalences of severe fatigue in neuromuscular disorders

Neuromuscular disorder	Prevalence of severe fatigue	Fatigue scale
Guillain-Barré syndrome[28, 38, 64, 78]	38-86%	FSS, FIS, VAS-F
Pompe disease[40]	67%	FSS
Myasthenia gravis[71]	82%	FIS, MFI
Post-poliomyelitis syndrome[53]	80%	NA
Facioscapulohumural dystrophy[48,81]	51-61%	CIS
Myotonic dystrophy[48,81]	53-74%	CIS
Charcot-Marie-Tooth disease type 1 (CMT type 1)[48,81]	55-64%	CIS

CIS=checklist individual strength; FIS=fatigue impact scale, FSS=fatigue severity scale; MFI=multidimensional fatigue inventory; VAS-F=visual analogue scale for fatigue; NA=not applicable.

population, used, assessment method and used definition of severe fatigue (e.g., cutoff point).

In the following section of this review we will discuss two different neuromuscular disorders for which fatigue has been reported as a important feature. GBS will be taken as an example of immune-mediated polyneuropathies and; Pompe disease as an example of metabolic myopathies.

Fatigue after Guillain–Barré syndrome (GBS)

GBS is an acute post-infectious immune-mediated polyneuropathy, characterized by rapidly progressive, symmetrical limb weakness and areflexia. Sensory disturbances, autonomic dysfunction and respiratory insufficiency often occur during its disease course. The severity of neurological deficits differs among patients and usually reaches a plateau phase within 2 weeks, which is indicative of the start of the recovery phase. Final outcome varies greatly among patients and ranges from severe residual weakness to complete recovery.[4, 99] CIDP may be considered a chronic variety of GBS.[98]

Patients experience fatigue already at the onset of GBS, and it can surprisingly persist for many years, even after total recovery of muscle weakness.[38] Fatigue in GBS was sporadically reported in the literature until 1999, when a cross-sectional case control study showed that fatigue is an essential and incapacitating residual symptom in patients with immune-mediated polyneuropathies.[64] Eighty percent of patients that recovered from GBS or CIDP or had ongoing CIDP suffered from severe fatigue. The patients stated that being severely fatigued leads to tremendous impairment in their daily life and social activities. As a consequence their quality of life was reported to be negatively affected in the long term. Some confounding factors could have affected the outcome of the study addressing fatigue in GBS. Sleep disorders, depression and level of physical activity were not systematically evaluated in the study. However, other studies confirmed the high prevalence of fatigue in GBS, being 38%,[78] 40%,[28] and 86%.[64] Part of the differences in estimated prevalence can probably be explained by differences in age between study

populations, as severe fatigue showed a positive correlation with age in GBS.[38] Severe fatigue does not seem to be related to clinical variables, antecedent infections or the severity of the neurological dysfunction in the initial phase of GBS,[38, 64] except that severe fatigue has a higher prevalence in female GBS patients (74% in females versus 45% in males, p=0.003). This difference in sex ratio is also seen in patients with chronic fatigue syndrome and can probably best be explained by endocrine differences between males and females.[77] A conventional nerve conduction study failed to show a relation between experienced fatigue and the occurrence of physiological fatigue/residual nerve dysfunction.[37] However, it could well be that newer or more advanced electrophysiological methods could be helpful to study this relationship. It has been added that CMAP scans potentially could be helpful to study this relationship.[24]

Another interesting finding was that severely fatigued GBS and CIDP patients showed a normal activity level, which was independent of their neurological condition. We presume that this neglect of patients' impaired load capacity may even increase symptoms of fatigue, as the actual performance of the patient does not match the patient's physical load capacity.[12] GBS not only affects the peripheral nerves, but also has an impact on psychosocial status. This may lead to a disturbed interplay between the structural and/or functional integrity of sensory, motor and cognitive symptoms, which may increase the sense of fatigue.

In general, the occurrence of fatigue in GBS seems independent of the severity of (residual) neurological deficits.[37, 38, 64] This differs from findings in other neuromuscular disorders, where severely affected patients experience higher levels of fatigue.[40, 49] The fact that GBS is an acute and monophasic disease may explain at least part of the differences found, as the other mentioned neuromuscular disorders are often chronic diseases and progressive in nature. In GBS and in CIDP it could well be that dysregulation of the hypothalamic–pituitary–adrenal axis accounts for residual fatigue, which could be linked to the antecedent infections seen in GBS and smoldering low-grade infections possibly going on in CIDP, because similar immune-mediated fatigue is seen as a residual symptom after an infectious illness.[46] Dysautonomia could also be an important contributor to fatigue[14] and is frequently seen in patients with GBS. At the moment further prospective studies in GBS are being executed (as part of the Dutch 'Graph study') to investigate the relation between fatigue in relation to dysautonomia, preceding infections, severity of disease and the occurrence of pain (L. Ruts, personal communication). In conclusion, we could state that peripheral fatigue is a more important contributor in the initial phase of the GBS. However, because feelings of fatigue persist after resolution of neurological symptoms, other factors must be responsible for fatigue in the long run. Post-infectious fatigue, dysautonomia and psycho-sociological consequences of the disease may be these causative factors.

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Fatigue in Pompe disease

Pompe disease (glycogen storage disease type II, acid maltase deficiency) is an autosomal recessive disorder caused by deficiency of lysosomal acid α-glucosidase. As a result, glycogen accumulates in lysosomes of several body tissues.[52] The disease presents as a spectrum of phenotypes. Patients with the classic infantile form are at the severe end of the spectrum. They suffer from a rapidly progressive disease course. Presentation is shortly after birth with hypotonia, feeding difficulties or respiratory problems. A hypertrophic cardiomyopathy is characteristically present. Patients usually die before the first year of life because of cardiorespiratory failure.[94] On the other end of the spectrum are children and adults with Pompe disease in which skeletal muscles are primarily affected. In these patients the disease is slowly progressive. The proximal, paraspinal and respiratory muscles are most involved. Patients may eventually become wheelchair bound and dependent on (nocturnal) ventilation.[42, 43]

Fatigue is a frequently experienced symptom in adults with Pompe disease and may have a disabling impact on the lives of patients.[40] Until recently, fatigue in Pompe disease did not receive much attention and was not structurally assessed. Incidentally fatigue had been reported in case reports and small patient populations of up to 16 subjects.[20, 62, 65, 85, 105] In a study on the natural course of Pompe disease in 54 patients, fatigue was rather unexpectedly reported to be the presenting symptom in 25% of the patients.[43] Seventy-six percent of the study population indicated that fatigue had an important impact on their daily life. Subsequently, fatigue was studied by using the FSS in more detail in an international group of 225 patients.[40] It was demonstrated that fatigue occurs in the majority (78%) of Pompe patients (FSS score ≥4) and severe fatigue (FSS score ≥5) in two-third of the patients.[40] The mean FSS scores differed significantly from healthy controls (mean FSS score of 5.2 versus 2.9, $p < 0.001$). Patients who did not use a wheelchair and respiratory support were significantly less fatigued when compared with patients using either or both ($p=0.01$).[40] It is of interest that Pompe patients reported only slightly lower levels of cognitive well-being in comparison with the general population.[41] This indicates that depression probably does not contribute to the observed prevalence of experienced fatigue, in contrast to, for example, multiple sclerosis, for which depression was found to have a substantial influence on the severity of fatigue.[74]

In Pompe disease the accumulation of glycogen in muscle fibres leads to structural changes of the muscle, which impairs muscle contraction, and in a more advanced state even destructs muscle fibers. This results in muscle weakness, muscle atrophy and muscle fatigability (peripheral fatigue). When patients are performing physical activities, they experience burning sensations of the proximal muscles and a heavy-legged feeling, which limits their force-producing capacity. In Pompe disease and other metabolic myopathies, such as McArdle's disease, muscle metabolism is altered, and certain me-

tabolites could impair skeletal muscle contraction through a direct action. This would be perceived as an increase in effort by the CNS, which then responds with down-regulating of activities to preserve homeostasis in the muscle.[69]

Respiratory dysfunction, due to diaphragmatic weakness and weakness of the respiratory muscles, is often present in Pompe patients and can also account for an increase in fatigue of central origin. Patients have to increase their respiratory effort to sustain adequate ventilation during exercise, or even already at rest. By feedback from the respiratory system (hypercapnia and hypoxia), fatigue is perceived at the CNS, and adequate ventilation is restored by decreasing the performed physical effort.[14, 69] Second, nocturnal hypoventilation leads to a decreased oxygenation of the brain and respiratory acidosis. Due to altered respiratory function the normal sleep pattern is disrupted;[63] as a consequence patients are not well rested, feel fatigued and suffer from day-time sleepiness during the day.

The pathophysiology of fatigue in Pompe disease still remains to be established, as this topic has not yet been investigated. Muscle fatigability of the proximal muscles plays an important role in Pompe patients, as they have difficulties in sustaining physical activities. Besides this localized form of fatigue, the occurrence of generalized fatigue is explained by the central governor model.[14, 69] Respiratory dysfunction also contributes to the level of fatigue perceived at the CNS.

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IMPLICATIONS FOR TREATMENT

Fatigue is a complex and multifaceted entity. In order to determine the treatment strategy for an individual patient, it is important to define the type of fatigue and the underlying factors that contribute. First, the implications of fatigue for the patient need to be investigated. This can be done by assessing the level of fatigue and its impact on daily activities and general wellbeing. Second, potential behavioral and psycho-sociological factors need to be identified that contribute to the experienced level of fatigue. Currently one or more of the following optional treatment strategies for fatigue in neuromuscular disorders can be used: (1) (symptomatic) treatment of the underlying disease, (2) rehabilitation and exercise, (3) pharmacotherapeutics directed to treat fatigue and (4) cognitive therapy. **Figure 1** shows how the different treatment strategies act on fatigue and the different domains of the WHO-ICF model.

Treatment of underlying disease and symptoms

When a patient complains of symptoms of fatigue, a diagnostic workup should be done to identify potential causes of fatigue. As mentioned, fatigue can be a symptom of vari-

ous (neurological) diseases, but also other underlying diseases may cause fatigue.[14] When the patient has already been diagnosed with a disorder that may explain fatigue, other causes like co-morbidity need to be ruled out. When possible, start or optimize treatment of the underlying disease, and eliminate other causal factors that may contribute to fatigue. For example, treatment of myasthenia gravis patients with adequate dosages of pyridostigmine can improve the clinical well-being and reduce feelings of fatigue.[39, 47] In a pilot study of late-onset Pompe patients treated with enzyme replacement therapy, the patients experienced a reduction in fatigue over a period of 8 years of treatment.[93] The experienced increase in energy level made them more independent in daily life and improved their participation in social activities.

Furthermore, in patients with neuromuscular disorders it is of utmost importance to rule out sleep-disordered breathing as a cause of sleepiness and fatigue during the day.[76] Nocturnal hypoventilation is frequently seen in neuromuscular patients with diaphragmatic weakness or in patients with obstructive apnoea or hypopnea. Besides hypoventilation, obstructive sleep apnea causes a disrupted sleep pattern, which results in a lowered quality of sleep.[86] Symptoms explained by nocturnal hypoventilation often herald complete respiratory failure by months or even years. Diaphragmatic weakness is often seen in Pompe disease and is present when there is a significant decline in pulmonary function when pulmonary function is measured in the sitting and the supine position.[96] A decline of 25% or more is called postural drop. Sleep-disordered breathing can be measured with sleep studies. When mechanical ventilation is implemented in patients suffering from nocturnal hypoventilation or sleep disordered breathing, symptoms of fatigue can be tremendously reduced.[76]

Rehabilitation and exercise

A well-fitted rehabilitation program can help patients to use their energy more efficiently. For example, implementation of ergo-therapeutic devices aimed at improving the ease of performing daily activities may increase the level of energy and thereby lessen symptoms of fatigue. Also training in developing coping strategies aimed at balancing mental and physical activities and rest may improve the patient's well-being. The potential effect of exercise programs on the level of fatigue was investigated in several studies.[12, 25, 34, 79] In patients recovered from GBS and in patients with ongoing but well-treated CIDP who suffer from severe fatigue, implementation of tailor-made exercise programs resulted in a reduction of 20% of self-reported fatigue. This improvement had been maintained at 2-year follow-up.[33, 34] These findings are consistent with results reported for healthy sedentary individuals (15%) and patients with multiple sclerosis (22%) after completion of an exercise program.[75, 80] However, the decrease in experienced fatigue in GBS after exercise therapy could not be explained by an increase in physical fitness.[12] Hypotheti-

cally, it could be that training also has a beneficial effect on the neuro-endocrine system and levels of neurotransmitters, which may reduce perception of fatigue. Additionally, recognition of the patients' complaints of fatigue and the beneficial effect of support from fellow patients within the training group are also potential factors that can reduce experienced fatigue.[12, 34] **Figure 3A,B** visualizes the relationships among training, physical fitness, activity and psychosocial factors in a model. **Figure 3A** shows the relationships as was hypothesized before the start of the training study and **Figure 3B** the remodeling after analysis of the results of the study.[12, 34]

Although strong evidence for a beneficial effect of exercise on experienced fatigue in neuromuscular disorder presently is relatively scarce, a low-intensity training schedule is likely to have a positive effect on fatigue and the general condition of patients with a neuromuscular disorder. Regular activity prevents physical deconditioning and muscle wasting. This was also shown in a rat model for post-poliomyelitis where physical activity reduced the size of the chronically enlarged motor units in denervated muscles.[91]

Pharmacotherapeutics

The efficacy of pharmacotherapeutic drugs such as amantadine, modafinil, albuterol and aminopyridine has not yet been convincingly proven for the treatment of fatigue in
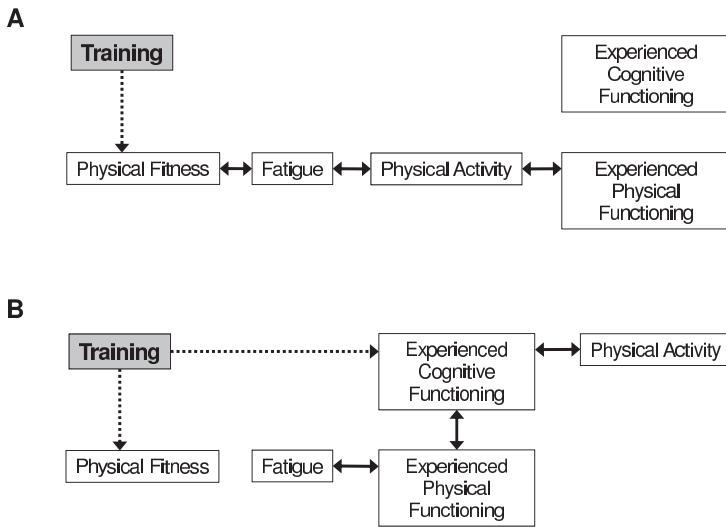
Figure 3 Model of fatigue in immune-mediated polyneuropathies
A Represents the hypothetical model of the mechanisms between the different domains in relation to physical training. **B** Is the model resulting from the correlations between the change scores of the different domains as a result of the training intervention. Thickness of lines between domains represents the strength of the relationship. Figures adapted from Bussmann, et al., Journal of Rehabilitation Medicine 2007.[12]

patients with neuromuscular disorders. Amantadine was shown to be effective in one-third to half of all treated multiple sclerosis patients.[15, 55, 67] Amantadine, however, did not show a beneficial effect in a well-powered randomized controlled trial on residual fatigue in patients who were in a good clinical condition after recovery from GBS.[36] In FSHD the effects of albuterol had no effect on experienced fatigue.[95] Modafinil has shown some beneficial effect in myotonic dystrophy; however, these were studies with small populations and a relatively short follow-up.[17, 61, 90, 103] Clinical trials studying the efficacy of pharmacotherapeutics as treatment for fatigue in other neuromuscular disorders are still lacking.

Cognitive behavior therapy

Patients' attitudes towards fatigue, coping mechanisms and psychosocial circumstances attribute to the level of experienced fatigue.[101] Patients may experience stress, depression and anxiety caused by disease-related social consequences, such as loss of employment, failure to fulfill family responsibilities and limitations in their social life. If necessary, patients can be supported with occupational rehabilitation. Cognitive behavioral therapy and psychological support have been shown to have a positive effect on experienced fatigue in cancer patients.[68] Whether the same results can be obtained in patients with neuromuscular disorders needs further investigation.

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CONCLUSION AND FUTURE LINES OF INVESTIGATION

The impact of fatigue on the lives of patients suffering from neuromuscular disorders has remained long unnoticed and has only recently been recognized as an important subject to be addressed in both clinical practice and research.[6, 14, 38, 40, 64, 104, 107] A high prevalence of fatigue was found in Pompe disease, GBS, post-poliomyelitis, hereditary polyneuropathy, myasthenia gravis and myotonic dystrophy.[38, 40, 48, 53, 64, 70] The prevalence of severe fatigue in these disorders was estimated to be similar to that in patients with multiple sclerosis.[50]

Fatigue can be subdivided into experienced fatigue and physiological fatigue. The severity of both experienced fatigue and physiological fatigue is influenced by psycho- sociological factors. In most neuromuscular disorders, but not in GBS as far as we know now, it also depends on disease severity.[38, 40, 49, 64, 100] The pathophysiologic mechanisms causing fatigue in neuromuscular disorders are not completely understood. Most likely there are two main pathways that lead to physiological fatigue: first, the pathology located in the PNS causes muscle fatigability by hampering muscle contraction, disturbance of muscle metabolism and inability to activate enough motor units (peripheral fatigue). Because

patients with neuromuscular diseases have to compensate for their loss of function by increasing their motor input to accomplish the same activities as a healthy person, the perceived level of effort at the CNS is higher, and patients become more easily fatigued. Additionally, the disease state of the muscles is continuously sensed at the level of the CNS, which will result in a constant feeling of fatigue of central origin, because body homeostasis needs to be maintained. Besides this flexible interactive feedback and feed forward system as described in the central governor model, other causes of central fatigue are proposed in patients with neuromuscular disorders.[81] Pathology of the PNS can eventually induce functional changes in the motor cortex, which may be a consequence of deconditioning and/or the relatively large demands put on the affected neuromuscular system.[16, 21, 60] This indirectly results in central fatigue due to CAF. Second, there is some evidence that pathology in neuromuscular disorders is not always restricted to the PNS and can affect the CNS as well. In FSHD and myotonic dystrophy, this was revealed using magnetic resonance imaging and somatosensory-evoked potentials.[26, 66] Although central fatigue usually correlates positively with the level of experienced fatigue, this correlation was not clearly found in 'neurologically well-recovered' GBS patients.[35, 81]

The differences of several aspects of fatigue found in GBS, as compared to other neuromuscular disorders, may lie in the fact that GBS is an acute and monophasic disease, often preceded by an infection. In contrast, the others neuromuscular diseases are often chronic and mostly progressive diseases.

For the management of fatigue in neuromuscular disorders, it is advised to start with optimizing treatment of the underlying disease and of possible co-existing causes of fatigue. When symptoms of fatigue persist, non-pharmacological interventions are preferable to pharmacotherapeutics, as pharmacotherapeutics have not yet been proven to be effective for the treatment of fatigue in neuromuscular disorders.[36] Further elucidation of the pathophysiology of fatigue, with special interest in the central governor model, may help to guide the development and proper targeting of new and hopefully effective pharmacotherapeutics. Meanwhile, the benefit of non-pharmacological treatments, such as low-intensity physical training, rehabilitation and cognitive behavioral therapy, as treatment options for fatigue in neuromuscular disorders needs further evaluation. The increasing awareness of fatigue as an important part of the burden of disease in neuromuscular disorders is not only important for the development of new treatment strategies, but also for improvement of current patient care.

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CHAPTER 4.2

A case of adult Pompe disease presenting with severe fatigue and selective involvement of type 1 muscle fibers

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ABSTRACT

We present a case of adult Pompe disease (acid maltase deficiency) with an uncommon clinical presentation characterized by severe fatigue and myalgia prior to the onset of limb-girdle weakness. Remarkably, the muscle biopsy demonstrated selective involvement of type 1 muscle fibers. The cause and clinical effects of fiber type specific involvement are currently unknown, but the phenomenon might contribute to the clinical heterogeneity in Pompe disease and the variable response to enzyme replacement therapy.

INTRODUCTION

Limb-girdle weakness is the most common and prominent presenting sign in adults with Pompe disease, an autosomal recessive metabolic disorder often referred to as acid maltase deficiency or glycogen storage disease type II (OMIM #232300). Pompe disease is a lysosomal storage disorder caused by the deficiency of acid α-glucosidase.[1-4] Expansion and malfunction of the lysosomal system followed by autophagosomal build-up leads to loss of muscle architecture and muscle function.[5-9]

The clinical spectrum of Pompe disease is very heterogeneous with regard to the age of onset, disease manifestations and rate of disease progression.[3,10] Light-microscopic examination of skeletal muscle from Pompe disease patients usually reveals a vacuolar myopathy and glycogen storage with nonselective involvement of the different muscle fiber types.[4] However, a limited number of cases have been reported showing preferential involvement of either type 1[11-13] or type 2 muscle fibers[14]. In all seven cases reported, the selective fiber type involvement was just reported as an unusual observation and it was not questioned whether patients with preferential glycogen storage in one specific fiber type might exhibit a different clinical phenotype.

We present a case of adult-onset Pompe disease with an uncommon clinical presentation characterized by severe fatigue and myalgia prior to the onset of limb girdle weakness. Remarkably, the muscle biopsy demonstrated involvement of only type 1 muscle fibers. This unusual observation is relevant in the context of recent publications suggesting that type 1 muscle fibers might respond better to enzyme replacement therapy than type 2 fibers.[15-17]

Case report

In January 2007, a 35-year-old Caucasian woman was referred to our hospital. Since August 2006 she suffered from severe fatigue and myalgia of the muscles of the shoulder girdle and the upper arms and limbs, especially notable when she walked the stairs and combed her hair. A few months later, she had noted minor weakness of the upper arms and legs. Because of these complaints she had abandoned her job as a children day-care worker. There were no clinical signs, such as feeling listless, being without energy, or a lack of motivation, suggesting a vital depression. Physical examination revealed no abnormalities. Neurological examination revealed no muscular atrophy or fasciculations. The muscles were not abnormally tender. Examination of the cranial nerves, sensory functions of the limbs and tendon reflexes were normal. There was symmetrical weakness of the shoulder girdle (m.deltoideus, m.infraspinatus), the gluteal muscles and the proximal muscles of the legs (m. iliopsoas, hamstrings), Medical Research Council (MRC) grade 4. The patient was able to squat, but used her hands to rise from the floor.

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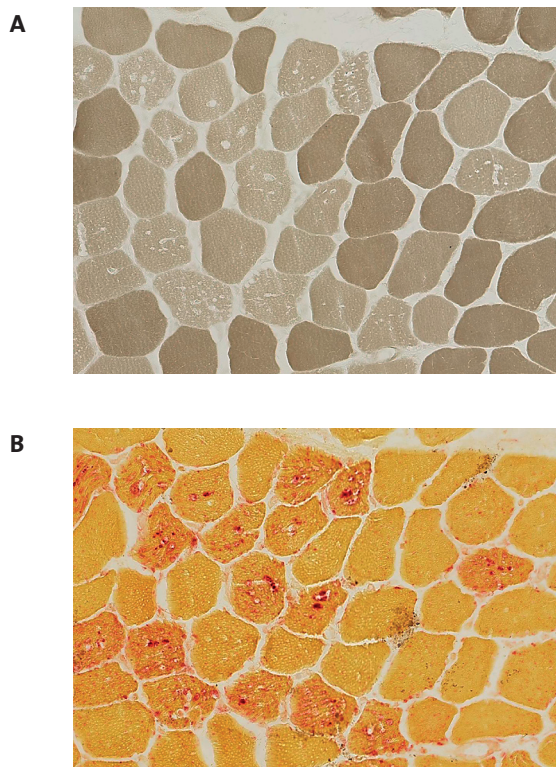
Figure 1 Biopsy from the m. quadriceps femoralis showing selective involvement of type I muscle fibers
ATPase staining at pH 9.4: Type 1 muscle fibers are lightly stained and are vacuolated (panel **A**). Acid phosphatase staining (red) of a serial section demonstrates lysosomal pathology in the type I muscle fibers (panel **B**).

Pulmonary function tests were normal. She had a mean score of 6.75 (range 0-7) on the fatigue severity scale (FSS), indicating severe fatigue.

Serum creatine kinase (CK) was elevated (1755 U/l; normal value <169 U/l). Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were slightly elevated. Erythrocyte sedimentation rate (ESR) and thyroid-stimulating hormone (TSH) were normal. Antinuclear antibodies (ANA), anti-SSA, anti-SSB and anti-Jo1, were negative (normal).

Based on the relatively short duration of her complaints, the presence of fatigue, myalgia, and the elevated CK, we considered it most likely that she would have an inflammatory myopathy. A muscle biopsy taken from the quadriceps muscle, however, revealed no signs of inflammation, but rather surprisingly showed a vacuolar myopathy solely affecting type 1 muscle fibers. The vacuoles stained positive for acid phosphatase. Rep-

resentative pictures are shown in **Figure 1**. Glycogen accumulation in some muscle fibers was detected by PAS staining and electron microscopy showed glycogen-filled vacuoles. No abnormalities were found in the NADH (nicotinamide adenine dinucleotide), SDH (succinate dehydrogenase), COX (cytochrome oxidase) and ORO (oil red O) staining. Based on these findings Pompe disease was suspected and subsequently confirmed by demonstrating acid α-glucosidase deficiency in leucocytes and cultured skin fibroblasts. In addition, DNA analysis revealed the presence of two pathogenic mutations in the acid α-glucosidase gene, c.-32-13 T>G and 525delT.

DISCUSSION

The case of Pompe disease described here is peculiar in that the patient presented with severe fatigue and myalgia prior to the development of limb-girdle weakness, and because fiber type involvement was restricted to the type 1 muscle fibers. Whether rapid progression, fatigue or pain is related to oxidative type 1 muscle fiber abnormalities in this patient is uncertain and has not been proven.

Although fatigue is prevalent in adults with Pompe disease, it is rarely reported as first symptom.[18] Fatigue can have many different causes.[19] In case of Pompe disease, expansion and dysfunction of the lysosomal system due to glycogen accumulation followed and accompanied by autophagic build-up destroys the muscle architecture and hampers the contraction.[20] Thus, it takes more energy to achieve the same power of contraction resulting in more rapid fatigue.[19,21] Decreased pulmonary function may also contribute to the level of fatigue, but our patient had a normal pulmonary function in both sitting and supine position. Muscle fiber type distribution varies widely within and between muscles depending on their function.[22] Therefore the selective involvement of type 1 muscle fibers in this case can be a chance finding and theoretically can be related to sampling differences, but might be related to the prominent fatigue, rapid progression or pain. Normally, type 1 muscle fibers are fatigue-resistant and well suited for prolonged aerobic exercise.[22] If type 1 fibers are selectively affected in the disease process, type 2 muscle fibers might be challenged to partially compensate for the loss of function while they are not suited for endurance. Whether these mechanisms explain the fatigue in our patient is as yet unknown. Perception of fatigue may also be related to non-physical causes.[23]

Selective type 1 muscle fiber involvement with vacuolization has previously been described in a limited number of cases of Pompe disease, but no discussion was devoted to its cause or clinical effects. Three of these five patients suffered from respiratory dysfunction, which is likely to cause fatigue, but pulmonary dysfunction was not found

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in the case we present. The muscle fiber type specific involvement however could be relevant in clinical practice because possibly it could be related to the effect of enzyme replacement therapy.

Research in mice showed that slow-twitch type 1 fibers respond well to ERT in contrast type 2 fibers. In particular, type 2b fibers seemed much more resistant to therapy. In knockout mice it was shown that the accumulation of autophagic vacuoles in skeletal muscle is limited to type 2 fibers. The combination of increased autophagic activity and inefficient endocytic trafficking in type 2 fibers may contribute to an incomplete therapeutic response.[15,24] However in a single patient with classic infantile Pompe disease it was shown that enzyme replacement therapy can reverse the pathological changes in both type 1 and type 2a muscle fibers.[17]

With this case report we want to draw attention to the occurrence of fiber type specific pathology in Pompe disease. It may be relevant for the clinical presentation and for the responsiveness to enzyme therapy, since it has been suggested, that type 1 fibers respond better to enzyme therapy than type 2 fibers.[17,24] Further research is required in adults with Pompe disease to draw further conclusions about the effect of fiber type specific involvement.

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CHAPTER 4.3

Enzyme replacement therapy and fatigue in adults with Pompe disease

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ABSTRACT

Background Pompe disease is a hereditary metabolic myopathy, for which enzyme replacement therapy (ERT) has been available since 2006. We investigated whether ERT reduces fatigue in adult patients with Pompe disease.

Methods In this prospective international observational survey, we used the Fatigue Severity Scale (FSS) to measure fatigue. Repeated measures ANOVA was used to analyze the data over time. In a subgroup of patients, we also evaluated muscle strength using the Medical Research Council scale, measured pulmonary function as Forced Vital Capacity, and assessed depression using the Hospital Anxiety and Depression scale

Results We followed 163 patients for a median period of 4 years before ERT and for 3 years during ERT. Before ERT, the mean FSS score remained stable at around 5.3 score points; during ERT, scores improved significantly by 0.13 score points per year (p<0.001). Fatigue decreased mainly in women, in older patients and in those with shorter disease duration. Patients' improvements in fatigue were moderately correlated with the effect of ERT on depression (ρ 0.55; CI 95% 0.07 to 0.70) but not with the effect of ERT on muscle strength or pulmonary function.

Conclusions Fatigue is a common and disabling problem in patients with early and advanced stages of Pompe disease. Our finding that ERT helps to reduce fatigue is therefore important for this patient population, irrespective of the mechanisms underlying this effect.

INTRODUCTION

Fatigue accompanies many chronic neuromuscular and neurological disorders,[1, 2] and is often reported by patients with Pompe disease,[3-5] an inherited metabolic myopathy caused by deficiency of acid alpha-glucosidase, a lysosomal enzyme. Pompe disease presents as a wide clinical spectrum, the most prominent symptoms in adults being muscle weakness and respiratory distress.[6, 7] As well as these main symptoms, many adults - however badly affected - complain of fatigue.[5]

The pathophysiology of fatigue in neurological disorders is not fully understood. As well as physiological changes in the muscle or the Central Nervous System (CNS), it may involve respiratory dysfunction and/or inadequate energy expenditure or energy production. Psychological fatigue ('weariness') may also be involved.[1, 2, 8]

At present there are no proven therapeutic strategies to combat fatigue. Its general management involves identifying and treating contributory factors such as psycho-sociological factors, sleep disturbances, and comorbidities.[1]

Since 2006, enzyme replacement therapy (ERT) has become available for Pompe disease. Though this has been shown to positively affect respiratory and muscle functions in adults,[9-13] very little is known about its effect on fatigue. While three studies suggested that ERT reduces fatigue in adult patients,[14-16] we do not know of any study that has investigated this subject in detail.

To establish whether ERT reduces fatigue, we therefore investigated a large international cohort of adult Pompe patients. We also investigated whether the potential effect of ERT on fatigue differed between subgroups of patients, and whether it was related to improvements or changes in muscle strength, pulmonary function, and/or depression.

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MATERIAL AND METHODS

Patients and settings

Data were collected between May 2002 and February 2011 as part of an ongoing observational follow-up study on the clinical course of Pompe disease in patients in Australia, Canada, Germany, the Netherlands, United States, United Kingdom, and in a small number of patients from other countries. Patients were recruited through national patient organizations or directly through our expertise center, the Center for Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center, as described previously.[3, 5] The study was approved by the Erasmus MC Ethical Committee, and all participants provided written informed consent.

Beyond a diagnosis of Pompe disease, there were no strict inclusion or exclusion criteria for participation in the study. For the analyses described in this paper, we included only patients aged 18 years and older who were receiving ERT, and who had had at least 6 months of follow-up before and after ERT.

Measurements

Each year, participants were asked to complete several questionnaires, including one on fatigue. Demographic and clinical data were collected on country of residence, age, year of diagnosis, gender, disease duration, and use of wheelchair and/or ventilator.

For patients seen at the Dutch center, more frequent measurements and additional data were available from clinical assessments and further questionnaires. As well as fatigue, these included pulmonary function and muscle strength (assessed between January 2005 and August 2009) and depression (assessed between January 2005 and February 2011) measured as described below.

Fatigue assessment

The severity and impact of fatigue were assessed using the Fatigue Severity Scale (FSS).[17] This self-report questionnaire focuses on the physical symptoms of fatigue, and measures the severity of fatigue and its impact on an individual's daily functioning. A mean score is calculated from the nine items, which range from 1 ('no signs of fatigue') to 7 ('most disabling fatigue'). Scores ≥4 indicate the presence of fatigue, and scores ≥5 severe fatigue.[17, 18] As described previously,[5] we used the English, Dutch and German translations. The FSS has demonstrated good internal consistency, reliability and validity in studies involving patients with several neurological disorders.[5, 17, 19, 20] When individual item scores were missing, the mean FSS score was calculated from the remaining items.[5] The maximum number of missing items per questionnaire was 2, and missing items were found in only 2% of the 1199 questionnaires completed.

Pulmonary function (Dutch patients)

Forced Vital Capacity (FVC) in sitting and supine positions was measured using spirometry as described previously.[21] Results were expressed as a percentage of the predicted normal value.

Muscle strength (Dutch patients)

Skeletal muscle strength was measured manually in scores from 0 to 5 using the Medical Research Council (MRC) grading scale.[22] A sumscore was calculated by adding the grades of 26 muscle groups as described earlier.[23] This sumscore could range from 0 (total paralysis) to 130 (normal strength), and was expressed as a percentage of the maximum

possible score of 130. When 3 or more muscle groups were missing the score was not calculated.[23]

Depression (Dutch patients)
Symptoms of depression were assessed using the depression subscale of the Hospital Anxiety and Depression Scale (HADS-D),[24] which ranges from 0 to 21. The HADS has been widely used in different disorders (including neuromuscular disorders), and has demonstrated good reliability and validity.[25]

Statistical Analysis
Analyses of the longitudinally assessed fatigue scores were performed using repeated measures ANOVA (random coefficient models), which allows for irregular measurement times. To assess the effect of ERT on the mean FSS scores, the model included linear effects of time before ERT and time after ERT. Per individual, the two segments connect at the time ERT started, a method generally known as the "broken-stick" method or "piece-wise linear regression". The two regression coefficients provide estimates of the mean annual change (slope) of the scores before and after the start of ERT. The difference between these two provides an estimate of the effect of ERT on the outcome measure.

For subgroup analyses, patients were divided into strata based on gender, age, disease duration, wheelchair use, and use of respiratory support, all at start of ERT.

For the Dutch patients we also analyzed the correlation between the effect of ERT on the FSS and its effect on the MRC sumscore, on FVC in upright and supine positions, and on the HADS depression score. After performing univariate analysis by using linear mixed-effects models with broken-stick evolutions for the FSS and the three other outcomes, we estimated random effects for each model using Empirical Bayes estimates (EB). Afterwards the correlation between the EB estimates for FSS and the other outcomes was calculated. The significance of the correlation coefficients was tested using 95% confidence intervals obtained from 1000 bootstrap samples.

Data were analyzed using SPSS for Windows (version 17, SPSS Inc., Chicago, IL) and SAS (version 9.2, SAS Institute Inc., Cary, NC). Bootstrap sampling was implemented in R (version 2.14). A p-value of ≤0.05 was considered statistically significant.

RESULTS

General characteristics
The eligibility criteria were met by 163 adults (55% female) out of a total of 383 patients participating in the survey. Thirty patients were excluded as they were younger than 18

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Table I Patient Characteristics at start of ERT

Characteristic	All (n=163)
Median age at start of ERT, years (range)	50 (24–76)
Median age at diagnosis, years (range)	37 (1–66)
Median disease duration, years (range)	13 (1–33)
Gender, no. (%)	
Female	90 (55)
Country of residence, no. (%)	
Netherlands	59 (36)
Germany	36 (22)
US	37 (23)
Other *	31 (19)
Use of wheelchair at start of ERT, no. (%)	
Yes	85 (52)
Respiratory support at start of ERT **, no. (%)	
Yes	81 (50)

*Continuous variables are expressed as median (range). Categorical variables are expressed as numbers (%). * Includes patients from Australia, Canada, United Kingdom and a small number of patients from other countries. ** Respiratory support includes partial and fulltime, invasive and non-invasive support.*

years of age, 67 were excluded for not receiving ERT, 85 had no follow-up measurements (yet), and 38 had less than 6 months follow-up before and/or after the start of ERT.

The 163 patients in our study (see **Table I**) had a median disease duration of 13 years. The patients' median age at start of ERT was 50 years (range 24–76 years); 52% used a wheelchair and 50% respiratory support. The median follow-up time before ERT was 4 years (range 0.5–8); after start of ERT, this was 3 years (range 0.5–8). Per patient, a median of seven questionnaires were completed (range 2–18).

At start of ERT, 85% of the patients with an available FSS score were fatigued (FSS ≥4) and 68% severely fatigued (FSS ≥5); at the last measurement, 79% were fatigued and 55% severely fatigued.

Change in fatigue scores before and during ERT

Before ERT, the mean FSS score remained stable (annual change of 0.01 score points; CI 95% -0.05 to 0.06; p=0.84). In contrast, the mean fatigue score declined significantly during ERT by 0.13 score points per year (CI 95% -0.19 to -0.07; p<0.001). Comparison of the trends in fatigue over time in the periods before and after the start of ERT showed that fatigue significantly improved during ERT (mean difference in slopes 0.14 FSS score points per year, 95% CI -0.23 to -0.04; p<0.01, **Figure 1**).

Relative to fatigue in the pre-treatment period, fatigue during ERT improved significantly in women, in older patients and in those whose disease duration was <15 years. This improvement was not statistically significant in men, younger patients, and those
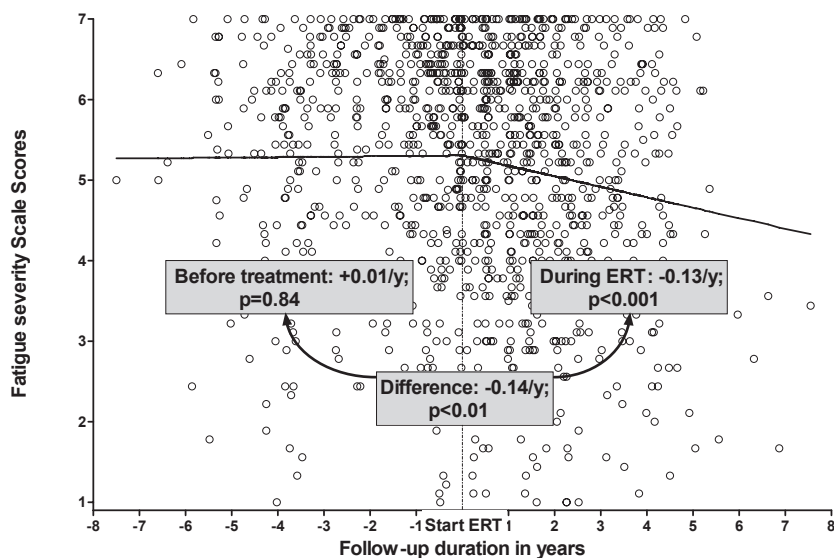
Figure 1 The figure shows the change in Fatigue severity-scale scores (FSS) before and after the start of enzyme replacement therapy (ERT)
The dots represent individual measurements and the lines represent the mean slopes calculated by the 'broken stick' repeated measures ANOVA for the group of 163 patients. The median follow-up during the natural course of Pompe disease was 4 years and the median follow-up during treatment was 3 years. The mean FSS score did not change significantly in the period before ERT, but declined significantly during ERT. The difference between the period before and during ERT was 0.14 FSS score points per year (p<0.01).

with longer disease duration (**Table II**). Fatigue improved significantly in patients who used a wheelchair and in those who were not on respiratory support. It also tended to improve in wheelchair-independent patients (p=0.06) and in those who received any kind of respiratory support (p=0.08). Statistical testing of the differences between these subgroups showed that these differences were not significant for any of the aforementioned subgroups.

Correlation between the effect of ERT on fatigue and its effect on muscle strength, pulmonary function and depression
For this part of the analysis, only the 59 Dutch patients were included. At start of ERT, their median age was 52 years (range 26–76), 59% were women and 46% used a wheelchair. Relative to the total study population, fewer patients used respiratory support (32% versus 50%) and more were fatigued at start of ERT (92% versus 85% of the total

Table II Subgroup analyses for the effect of ERT on the fatigue severity-scale scores

Subgroups	Difference in FSS score before and during ERT * Score points/year (95% CI)	p-value **
All	-0.14 (-0.23 to -0.04)	<0.01
Gender		
Female (n=90)	-0.18 (-0.31 to -0.05)	<0.01
Male (n=73)	-0.08 (-0.23 to 0.07)	0.30
Age at ERT, years		
< 45 (n=66)	-0.10 (-0.27 to 0.07)	0.26
≥ 45 (n=97)	- 0.15 (-0.27 to -0.03)	0.01
Disease duration, years		
< 15 (n=94)	-0.19 (-0.32 to -0.05)	<0.01
≥ 15 (n=68)	-0.07 (-0.22 to 0.07)	0.33
Wheelchair use		
Yes (n=85)	-0.13 (-0.24 to -0.01)	0.04
No (n=78)	-0.15 (-0.32 to 0.01)	0.06
Respiratory support *		
Yes (n=81)	-0.13 (-0.27 to 0.02)	0.08
No (n=82)	-0.14 (-0.27 to -0.004)	0.04

*Data show the mean changes in score points per year (sp/y) as calculated by stratified analysis using repeated measures ANOVA; CI = Confidence Interval; FSS = Fatigue Severity Scale. * Comparison between change over time in FSS before and during ERT. ** P-value for the difference in FSS score before and during ERT per subgroup. Statistical testing of the differences between these subgroups showed that these differences were not significant. *** Respiratory support includes partial and fulltime, invasive and non-invasive support.*

patient population). Median MRC sumscore (in percentage) at start of ERT was 78% (range 48–92). Median FVC percentages were 70% in sitting position (range 11–107) and 49% in supine position (range 23–99). Only 13 patients (22%) scored ≥8 on the HADS depression subscale, indicating clinical signs of (borderline) depression. Median HADS depression score was 4 (range 0–15).

As in the total study population, FSS scores significantly decreased during ERT. The difference with the pre-treatment period was borderline significant (**Table III**), and was moderately correlated with a decrease in the level of depression (ρ 0.55; CI 95% 0.07 to 0.70). No significant correlations were found between the improvement in fatigue and muscle strength in response to ERT and in pulmonary function in upright and supine positions.

DISCUSSION

This is the first prospective follow-up study to assess the effect of ERT on fatigue in a large number of adult Pompe patients. We found that ERT significantly reduces self-reported

Table III Change in fatigue as a result of ERT and its correlation with the change over time in muscle strength, pulmonary function and level of depression.

	Change over time			
	Before ERT (95% CI)	During ERT (95% CI)	Difference before and after ERT (95% CI)	Correlation to FSS * ρ (95% CI)
Main outcome measure				
FSS (n=59)	-0.04 (-0.13 to 0.04)	-0.21 (-0.33 to -0.09) **	-0.17 (-0.35 to 0.01)	Reference
Covariates				
MRC sumscore (n=55 ***)	-1.28 (-1.80 to -0.49) **	2.01 (1.20 to 2.81) **	3.29 (2.04 to 4.53) **	-0.36 (-0.61 to 0.08)
FVC upright position (n=52 ***)	-1.95 (-3.09 to -0.83) **	0.06 (-1.09 to 1.21)	2.02 (0.26 to 3.77) **	-0.26 (-0.49 to 0.08)
FVC supine position (n=45 ***)	-1.71 (-2.76 to -0.67) **	-0.67 (-1.79 to 0.45)	1.04 (-0.50 to 2.57)	-0.31 (-0.56 to 0.17)
HADS depression (n=59 ***)	0.08 (-0.40 to 0.48)	-0.48 (-0.85 to -0.11) **	-0.57 (-1.23 to 0.09)	0.55 (0.07 to 0.70) **

*Data show the mean changes in score points per year (sp/y) as calculated by univariate analysis using linear mixed-effects models CI = Confidence Interval; FSS = Fatigue Severity Scale; MRC = Medical Research Council; FVC = Forced Vital Capacity; HADS = Hospital Anxiety Depression Scale. * Correlation between the differences in FSS scores and the difference in the other outcome measures following ERT. ** Represents statistical significance. *** Only patients with sufficient data were included*

fatigue, the mean decrease in FSS score being 0.14 per year relative to the pre-treatment period. The decrease in fatigue during ERT was correlated with improvements in depression, but not significantly with changes in muscle strength or pulmonary function. The effect of ERT on fatigue was not consistent across patient subgroups: fatigue decreased mainly in women, older patients and those with shorter disease duration.

Our study mirrors previous findings in this cohort, providing a reminder that fatigue is a highly prevalent symptom among adults with Pompe disease. Therapies that can reduce fatigue in this population are therefore of great importance, and our finding that ERT positively affects fatigue is a significant finding. While the annual improvement might seem small (0.14 points per year), it resulted in a substantial drop in the proportion of patients who were fatigued or severely fatigued at the end of our follow-up.

Subgroup analyses suggest that fatigue may be more responsive to ERT in women, older patients and those with shorter disease duration. A better response in women might be explained by the more pronounced effect of ERT on muscle strength in females that was identified recently by our group.[23] It is unknown whether hormonal influences are involved in this. The better effect in patients with shorter disease duration might be due to less severe muscle damage. The absence of an effect in younger patients may seem at odds with this, but it is possible that younger patients have more demanding lifestyles, resulting in more fatigue. Since glycogen degradation occurs mainly in the cy-

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toplasm, we doubt that lower levels of fatigue can be attributed to the increased release of glucose from the lysosome (by ERT mediated lysosomal glycogen degradation).[26]

Muscle strength, pulmonary function and depression, have been shown to be related to fatigue in other neurological disorders.[1, 2, 8] Against our expectations, we found no significant correlation between the improvement in fatigue and in muscle strength or pulmonary function. This might be due to the smaller sample size available for this sub-analysis. Alternatively, it may be that the level of fatigue is determined by the degree of muscle endurance rather than of muscle strength.[27] Because patients with impaired pulmonary function often use mechanical ventilation, their oxygen levels will be normal, which could explain the absence of a correlation between the response to ERT in fatigue and pulmonary function.

We found that as fatigue decreased during ERT, the scores on the depression scale also decreased. Several studies have described a relationship between depression and fatigue in neurological disorders.[2, 28-31] Depression may predispose for fatigue,[2, 28] but fatigue secondary to an underlying illness can also cause depression, with one sometimes influencing the other.[29] Since the majority of patients (78%) were not depressed, ERT has presumably contributed to a decrease in fatigue by affecting the underlying pathophysiology

As fatigue is a multifactorial entity, it is likely that other factors – including changing patient's perspectives and perceptions, intensified medical care and altered muscle metabolism – contribute to its improvement after ERT. Although, as a treatment of the underlying disease, ERT seemed to reduce fatigue, it is hard to define the extent to which it acts directly (by reversing disease-related pathophysiology) or indirectly (through psycho-sociological factors). Further research is needed to unravel the underlying mechanisms.

This study benefits from the relatively large number of patients who participated, and from the fact that patients were included irrespective of their disease severity; the study thereby represents the entire spectrum of adult Pompe disease. Despite the large sample, it was not possible to build multivariate models, and multiple testing might limit the results of the subgroup analysis. Our correlation analysis was based on a subset of patients and was limited by a smaller sample size.

Fatigue is a subjective and complex concept that is difficult to define and measure. The FSS is a uni-dimensional scale that measures fatigue as a single construct. Because of its brevity and simplicity, we preferred the FSS to a multi-dimensional scale in which different forms of fatigue – such as physical, cognitive and psychosocial fatigue – are assessed separately. Uni-dimensional and multi-dimensional scales have been found to produce similar measurements of fatigue.[32]

CONCLUSIONS

Fatigue is a common problem in patients with Pompe disease. Our finding that ERT helps to reduce it is therefore important for this patient population, irrespective of the mechanisms underlying this effect. Fatigue decreased mainly in women, in older patients and in patients with short disease duration.

To manage fatigue successfully, treatment options such as rehabilitation and exercise [33] should be considered in addition to ERT. Further investigations should be devoted to the roles of pharmacotherapeutics and cognitive therapy in treating fatigue, and to the exact role of muscle cell changes, pulmonary function, and psychological and other factors that may be associated with it.

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CHAPTER 5

General Discussion

Through the advent of Enzyme Replacement Therapy (ERT) using alglucosidase alfa (Myozyme®), Pompe disease has become the first treatable hereditary neuromuscular disorder. This has changed the perspectives for patients affected by Pompe disease for the better. In 2006, at the time alglucosidase alfa was registered as a treatment for all forms of Pompe disease, data on treatment efficacy in children and adults were still limited. In anticipation of the registration, we started to systematically collect information on the clinical manifestations and the natural course across the clinical spectrum; from 2004 onwards, in a nationwide prospective study [**Appendix A**]. The same set of standardized measurements was used before and after the patients started to receive ERT enabling us to evaluate the change in the course of disease caused by ERT for each individual patient.

With the implementation of this new treatment, a number of questions arose, such as 'which factors are predictive of a good response?; 'what is the clinical impact of antibodies on therapeutic efficacy?'; 'are there any risks for the mother and her (unborn) child to continue treatment with alglucosidase alfa during pregnancy and lactation?; and 'does treatment with ERT decreases the burden of fatigue in adults with Pompe disease?'. The studies described in this thesis aimed at answering these questions.

This general discussion summarizes the most important findings and compares these with what is known from the literature. The results are translated into recommendations that can be used in clinical practice. At the end of this chapter some methods are addressed that might improve the effect of ERT and potential new treatments are discussed.

THE CLINICAL SPECTRUM AND NATURAL DISEASE COURSE IN ADULT POMPE DISEASE

The clinical spectrum

The Dutch patient cohort of 94 patients (including three patients from Belgium) described in this thesis [**Chapter 2.1**], reflects the remarkable broadness of the clinical spectrum with regard to the wide range in age at first clinical presentation, heterogeneity in disease manifestations, and differences in the rate of disease progression as described in the medical literature.[1-9] In this cohort, all but one patient carried the c.-32-13T>G (IVS-1) mutation in the one allele in combination with a 'null' mutation in the other allele. In these patients, age of symptom onset ranged from 1 to 62 years and age at diagnosis from 1 to 63 years. Our findings were thereby very similar to those of a retrospective study. This retrospective study reviewed 225 cases published in the literature.

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Among these cases the maximum age for onset of symptoms was 68 years and for age at diagnosis 71 years.[8]

The advantage of our study approach over clinical studies that either included small numbers of patients or used strict eligibility criteria, is that the nation-wide Dutch cohort is unbiased in the sense that it included almost all Dutch patients with a confirmed diagnosis of Pompe disease, from asymptomatic to very severely affected. However, there is also a draw-back in that almost all our patients carried the c.-32-13T>G (IVS1) mutation so that the extrapolation of our findings to patients not carrying this mutation must be done with caution.

Diagnostic delay

The rarity of Pompe disease, its heterogeneous clinical presentation, and the overlap of signs and symptoms with other neuromuscular disorders may initially result in a low clinical suspicion causing diagnostic delay.[5 8 10-13] In our cohort, there was on average a seven-year delay between the time that the first Pompe related-symptoms were noted and the actual time of diagnosis. The diagnostic delay was no different than reported in the literature review of 225 cases published in 2005.[8] When we examined the length of the diagnostic delay before and after the registration of ERT, surprisingly the median delay in the Netherlands had doubled from a median of 5 years before 2006 to a median of 10 years thereafter. It is also interesting to note that the number of diagnoses made in the Netherlands doubled, from three per year between 1990 and 2006 to 6-7 per year after the introduction of ERT in 2006. These trends may well be explained by two factors. First, when ERT became available, neurologists probably screened for Pompe disease among all their patients with limb-girdle weakness who did not have a sufficient diagnosis. Second, since the introduction of ERT ongoing efforts are made to increase the awareness for Pompe disease. Through this, neurologists are better able to recognize patients across the entire clinical spectrum and also test for Pompe disease in patients presenting with less familiar features, such as ptosis or scapular winging, now associated with the disease.

Since the majority of patients experience substantial disability at the time of diagnosis, there is potentially much to be gained by shortening the gap between onset of symptoms and diagnosis followed by a timely start of ERT.[14] For shortening the diagnostic delay, it is important to identify the responsible factors first. One such factor, as shown by data from the (industry driven) international Pompe registry, is that patients presenting with less familiar manifestations of Pompe disease are diagnosed later than patients presenting with typical signs or symptoms like respiratory or musculoskeletal problems.[15] Our finding that some of the less well-known features of Pompe disease are far more common than initially thought, might contribute to the timely diagnosis of

patients presenting with these symptoms [**Chapter 2.1**].[16] For instance, we found that ptosis (mainly asymmetrical) occurred in 23% of the patients, scapular winging in 33%, bulbar muscle weakness in 27%, and scoliosis in 23%.

Limb-girdle and respiratory muscle weakness

The first presenting symptoms of children and adults with Pompe disease are most frequently related to limb-girdle weakness. The pattern of muscular weakness, as evaluated by manual muscle testing using the Medical Research Council (MRC) grading scale and quantitative muscle testing using Hand-Held Dynamometry (HHD), is symmetrical and reflects the typical limb-girdle distribution. The distal muscles of the upper and lower extremities are relatively spared, and if they are affected at all, this occurs only late in the disease course [**Chapter 2.1**].[16] Our findings on clinical examination are in accordance with the pattern of muscle involvement as revealed by Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) scanning.[17-19]

The majority of Pompe patients have also respiratory insufficiency with diaphragmatic weakness, as 60% of the patients have an abnormally low Forced Vital Capacity (FVC) in upright seated position and 80% of the patients have an abnormal FVC in supine position [**Chapter 2.1**].[16] It was striking that two of the patients we have seen had a normal pulmonary function in upright seated position, but required night-time mechanical ventilation due to disproportional diaphragmatic weakness. This stresses the need for also performing spirometry also in the supine position as a standard screening measurement in all patients diagnosed with Pompe disease, as otherwise the development of respiratory failure may be discovered too late.[20]

Unexpectedly and remarkably, the extent of pulmonary dysfunction was more pronounced in males than in females.[20] At study entry 38% of the males against 17% of the females used mechanical ventilatory support, while 13% of the males and none of the females used invasive ventilation.[16 20] These findings are consistent with a German study in 38 patients, in which 54% of the males and 20% of the females used mechanical ventilatory support at study entry [5] In our study, there were no differences in severity of limb-girdle weakness, disease duration or age between males and females that could clarify this gender difference. It was also found that males have a higher susceptibility to sleep-disordered breathing than females. With regard to this finding, it was speculated that it could be explained by the higher upper airway resistance in males compared to females caused by a different body-fat distribution and differences in sex hormones induced central ventilator control.[21 22] However, this has not been proven as yet for Pompe disease.

Main findings on the clinical features of adults with Pompe disease
- The broadness of the clinical spectrum of Pompe disease is reflected in the Dutch patient cohort.
- The pattern of muscle weakness generally has a typical limb-girdle distribution.
- Respiratory insufficiency with diaphragmatic weakness is present in 60% of the patients while seated and in 80% while supine.
- Males have more severe pulmonary dysfunction than females.
- Less familiar features of Pompe disease, such as ptosis (23%), scapular winging (33%), bulbar muscle weakness (27%), and scoliosis (23%) are more common than formerly apprehended.

Recommendations for clinical practice
- It is important to increase the awareness for Pompe disease by spreading the knowledge of the wide clinical spectrum and clinical manifestations. This helps to shorten the diagnostic delay, and is likely to decrease the burden of Pompe disease.
- Patients should be screened for respiratory insufficiency by performing pulmonary function testing in both upright as well as in supine position to warrant a timely initiation of respiratory support measures.

Natural course

When the prospective observational study started, we opted for a follow-up period of at least two years for each patient before the start of ERT. This however was no longer feasible with the introduction of alglucosidase alfa (ERT) in 2006. Especially for patients who were severely affected, had pronounced pulmonary involvement, and/ or deteriorated rapidly, ERT was started as soon as possible. As a consequence, the length of pre-treatment follow-up for these more severely affected patients was shorter than originally planned, whereas the less severely affected patients had a longer pre-treatment follow-up. At the end, the final median follow-up became 1.6 (0.5 – 4.2) years, which is rather short for a slowly progressive disease, although it is the longest follow-up among the published prospective natural course studies in untreated adult Pompe disease (**Table I**).

During follow-up, muscle strength and pulmonary function declined gradually [**Chapter 2.1**].[16] These findings compare to rates of decline reported by other studies (**Table I**). It seems that the patients in the study by Wokke *et al.* showed a faster decline during the 12 month follow-up period, however this is largely explained by reporting relative instead of absolute percentages as change from baseline.[9] Although the average yearly decline appears rather small, the impact on disability over the years is substantial: 15 years or more after disease onset, 70% of the patients are wheelchair dependent and 60% require some form of mechanical ventilation.[2] Thus, the severity of disease is clearly related to disease duration.[2 23]

About 15% of the patients remained fairly stable during follow-up, relative to the total group these patients had a shorter disease duration (7.3 versus 15.5 years, *P* = 0.03) and

Table I Overview of clinical studies on the natural course of disease in children and adults with Pompe disease

	Study			Muscle strength & function[a]					Pulmonary function[a]		
	Patients (No.)	Follow-up (Year (range))	Study design	MRC sumscore (%/year)	HHD sumscore (%/year)	QMT score (%/year)	6 MWT (meters/year)	(F)VC upright (%/year)	(F)VC supine (%/year)	MEP (%/year)	MIP (%/year)
Van der Beek, et al. 2009 [33]	16	16 (4-29)	Retrospective	-1.3 (p<0.001)	NR	NR	NR	-1.6 (p=0.002)	NR	NR	NR
Wokke, et al. 2008 [9]	58	1	Prospective	NR	NR	Arm -4.0[b] (p<0.01) Leg -7.1[b] (p<0.01)	NR	-4.6[b] (p<0.01)	-5.5[b] (p<0.01)	-7.3[b] (p<0.01)	no change
van der Ploeg et al. 2010 [29]	30	1.5	RCT (placebo arm)	NR	NR	Arm 0.5 (p=0.11[c]) Leg -1.3 (p=0.19[c])	-2.0 (p=0.03[c])	-1.5 (p<0.01[c])	NR	-0.4 (p=0.04*)	-0.3 (p=0.09*)
Van der Beek, et al.2011 [20]	53	1.6 (0.5 – 4.2)	Prospective	NR	NR	NR	NR	-0.9 (p=0.094)	-1.2 (p<0.05)	-3.8 (p<0.01)	-3.2 (p=0.02)
Van der Beek, et al. 2012 [16] [Chapter 2.1]	66	1.6 (0.5 – 4.2)	Prospective	-1.3 (p<0.001)	-2.6 (p<0.001)	NR	NR	-1.0 (p=0.06)	-1.3 (p=0.02)	NR	NR
de Vries / van der Beek et al. 2012 [36] [Chapter 3.1]	49	1.2 (0.3-2.8)	Prospective	-1.2 (p<0.001)	-2.8 (p<0.001)	NR	NR	-2.0 (p=0.001)	-1.8 (p=0.002)	NR	NR

No. = number; MRC = Medical Research Council; HHD = Hand-Held Dynamometry; QMT = Quantitative Muscle Testing; 6MWT= Six-Minute Walk Test; (F)VC = (Forced) Vital Capacity in percentage of the predicted normal value; MEP = Maximal Expiratory Pressure; MIP = Maximal Inspiratory Pressure.

[a] Reported percentages or percentages of the predicted normal value are expressed in absolute change from baseline unless otherwise specified. [b] Reported percentages of the predicted normal value are relative changes as compared to baseline. [c] Change from baseline for the placebo arm of the LOTS; p-value for the difference between the treatment arm and the placebo arm.

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were less severely affected at baseline, although differences for the latter did not reach statistical significance. In about 10% a more rapid deterioration was observed for either muscle strength or pulmonary function. Factors that predicted a faster decline in muscle strength included a longer disease duration (>15 years) and more distinct pulmonary involvement (FVC in seated position ≤80% of the predicted normal value). Wokke *et al.* also found that patients who had more advanced limb-girdle weakness and pulmonary dysfunction at study entry declined more rapidly over the 12-month study period.[9]

Van der Beek *et al.* showed that in the cross-sectional study on respiratory status in the Dutch patient cohort longer disease duration, male gender and patients with advanced disease had a worse pulmonary status. However, during prospective follow-up we could not detect prognostic factors related to decline in pulmonary function. Not finding these prognostic factors can probably be explained by the aforementioned differences in length of the pre-treatment follow-up between the more and the less severely affected patients. As a result, the average yearly decline in pulmonary function was less pronounced, and prognostic factors for respiratory outcome were more difficult to establish.

From the patients' perspective it is most important how Pompe disease interferes with their daily life and how treatment improves their physical functioning. The Quick Motor Function Test (QMFT; see also the paragraph on outcome measures) measures several domains of physical functioning.[24] The test correlates very well with scores of proximal muscle strength (MRC and HHD), but unfortunately did not measure changes during prospective follow-up. This could be due to: I) the limited duration of follow-up (in a slowly progressive disorder), II) the unbalanced difference in the length of pre-treatment follow-up between severely and less severely affected patients, and III) a learning effect (improvement of performance on repetitive testing) during the first period of follow-up.

We estimated the natural disease course in a cohort of patients diagnosed with Pompe disease, who were referred to our center. When interpreting these natural course data, one should realize that we have to do with the 'iceberg phenomenon' (*John M. Last*, The Lancet 1963). This selection bias can be overcome by carrying out a population-based study.

Survival

With respect to long-term disease outcome, our group was the first to study survival in adults with Pompe disease [**Chapter 2.2**].[25] Survival is a frequently used outcome measure in classic-infantile Pompe disease, since the majority of untreated patients succumbs in the first year of life. This is quite different in non-classic forms of Pompe disease wherein some diagnoses are made beyond the sixth decade of life. The exceptionally long follow-up of patients (since 2002) participating in the IPA/Erasmus MC Pompe Survey has cre-

ated the opportunity to compare the life expectancy of adults with Pompe disease and adults in the general population. The mortality rate of the patients turned out to be 2-3 times higher. Predictors of the higher mortality rate were wheelchair use, mechanical ventilation and a high level of disability as measured with the Rotterdam-Handicap Scale.

Considerations regarding outcome measures in adult Pompe disease

The clinical studies summarized in **Table I** and **Table III** show the wide variety of outcome measures that were used in Pompe disease, particularly with regard to muscle strength and muscle function. For international comparison of data, it is important that outcome is measured in a standardized way using the same set of outcome measures. At the ENMC workshop of 2010 consensus was reached about a minimal set of assessments for adult Pompe patients. The set must include at least the six-minute walk test (6MWT), manual muscle strength testing, pulmonary function tests (FVC), and functional tests (Walter Gardner scale (WGM)) and timed tests).[26]

For measuring the muscle strength our group has chosen for the MRC grading scale and HHD scores. Both methods have their limitations when used for measuring changes in muscle strength in mildly affected patients, as in the higher regions the score is more dependent on the strength of the examiner. For HHD the maximal muscle strength was defined as the median strength of the general population, meaning that obtained results above the median were rounded to the median muscle strength of respectively healthy women or men. We defined maximal muscle strength in this way, for the reason that the average examiner cannot reliably measure forces beyond 250 Newton.[27] The advantage of HHD over the MRC grading scale is that it measures muscle strength in a quantitative and linear way.

To overcome the non-linearity of the MRC sumscore, a revised MRC scoring system was recently developed on the basis of Rasch methodology.[28] This methodology transforms an ordinal score into a linear measure and is based on the assumption that patients who are less affected should have a greater chance of getting a better score. However, it still needs to be determined in clinical practice whether this Rasch-build MRC sumscore is a better way of measuring the course of muscle strength in patients with Pompe disease.

A better method for measuring muscle strength in mildly affected patients might be Quantitative Muscle strength Testing (QMT) as applied by Wokke *et al.*[9] and in the Late-Onset Treatment Study (LOTS).[29] As compared to MRC and HHD, this method measures more precisely differences in muscle strength in milder affected patients and is not dependent on the strength of the examiner. However, QMT has several disadvantages: I) it is a time consuming test, II) the test is limited to certain skeletal muscle groups, and III) requires the installation of specialized equipment, making QMT unattractive for use in daily clinical practice.

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To all methods of assessment described here, it applies that these are not well suitable for measuring the strength of the trunk muscles, which are the first to be affected in the disease process.[16][17][19] Early detection of trunk musculature weakness is of clinical importance as this signals that the patient is becoming symptomatic.

With regard to physical function testing, a frequently used outcome measure is the Walter Gardner scale (WGM). This is a crude measure ranging from 0 (the patient has a normal activity pattern) to 10 (the patient is bedriddenn). **Table I** and **Table III** show that the average WGM score did not change over time in most studies, indicating that this scale has limited responsiveness. Another commonly used test is the six-minute walk test, which is considered to be a reliable outcome measure. The six-minute walk test is also used in other neuromuscular disorders, for example in Duchenne muscular dystrophy.[30][31] However, because the test was originally designed for patients with chronic bronchitis,[32] it does not specifically measure the limitations of patients with limb-girdle weakness. Moreover, this does not particularly mean that minor changes on this assessment scale imply important changes for the patients in daily life.

As none of the tests was truly ideal, we developed the Quick Motor Function Test (QMFT) [**Appendix C**] for evaluating the impairment in physical functioning for patients with limb-girdle weakness.[24] This test was constructed on the basis of: I) the Gross Motor Function Measure (GMFM); [33][34] II) expertise of clinicians involved in the care of patients with Pompe disease; and III) on patient-reported information gathered via the IPA/Erasmus MC Pompe Survey.[3][35] As mentioned before, we could not demonstrate a change during the pre-treatment period, but after start of ERT the average QMFT increased for the total group (0.7 pp/y, $P = 0.14$), with significant increases observed in the milder affected patients (2.1 pp/y, $P = 0.01$) [**Chapter 3.1**].[36]

Main findings on the natural course in adults with Pompe disease
- For most patients the disease evolves gradually and slowly. Some patients remain fairly stable for a long time, others suffer from more rapid deterioration.
- Longer disease duration and distinct pulmonary involvement are usually associated with a fast decline in muscle strength.
- The average life expectancy of untreated adult patients is shorter than that of the general population.

Recommendations for clinical practice
- The advised minimal set of assessments for adults with Pompe disease should preferably include the six-minute walk test, manual muscle strength testing, pulmonary function tests ((F)VC) in upright seated and supine position, and functional tests.[26]
- Assessment scales preferentially should have good clinimetric properties, such as those based upon Rasch analysis.

Fatigue

In recent years, the burden of fatigue in patients with Pompe disease and other neuromuscular diseases has gained more attention. In Pompe disease 24% of the patients reported fatigue as an initial symptom and 76% indicated that fatigue had an important impact on their daily life.[3] In two subsequent publications of the IPA/Erasmus MC Pompe Survey 78-85% of Pompe patients reported to be fatigued (Fatigue Severity Scale (FSS) ≥4) and 67-68% severely fatigued (FSS ≥5) [**Chapter 4.3**; **Appendix B**].[37 38] The reported prevalence rates in Pompe disease and other neuromuscular diseases resemble those reported for multiple sclerosis (**Table II**) [**Chapter 4.1**].[39-49]

In many neurological disorders[46 50-53] such as Pompe disease,[38] the prevalence as well as the severity of fatigue partly depends on disease severity. An exception is formed by fatigue in Guillain-Barré syndrome (GBS), which may be due to the fact that this is an acute and monophasic disease, often preceded by an infection.[45 46] Most patients with GBS experience long-lasting severe fatigue, which in part seems to be caused by axonal degeneration of the peripheral nerves.[54]

In Pompe disease the pathophysiological mechanisms causing fatigue are not fully understood, although it seems plausible that the muscle pathology leads to an increase in muscle fatigability (peripheral fatigue) [**Chapter 4.1**].[48] In McArdle disease, another metabolic myopathy, alterations in muscle metabolism impair skeletal muscle contraction.[55] However, in Pompe disease the skeletal muscle glycogenolytic capacity is not impaired, as shown in a recent publication, and the reduced exercise capacity is more likely the result of muscle weakness and wasting.[56] Moreover, as the limitations in muscle performance are perceived by the central nervous system (CNS), it is hypothesized that the CNS generates a constant feeling of fatigue (central fatigue) that initiates down-regulation of activities. This may also protect the body from damage due to excessive exercise (central governor model).[57]

Table II Severe fatigue in neuromuscular disorders

Neuromuscular disorder	Prevalence of severe fatigue	Fatigue scales
Pompe disease [**Chapter 4.3**] [37 38]	67-68%	FSS
Guillain-Barré syndrome (GBS) [43-46 49]	38-86%	FSS, FIS, VAS-F, R-FSS
Myasthenia gravis [42]	82%	FIS, MFI
Post-poliomyelitis syndrome [41]	80%	NA
Facioscapulohumural dystrophy (FSHD) [39 40]	51-61%	CIS
Myotonic dystrophy (DM) [39 40]	53-74%	CIS
Charcot-Marie-Tooth disease type 1 (CMT type 1) [39 40]	55-64%	CIS

CIS=Checklist Individual Strength; FIS=Fatigue Impact Scale; FSS=Fatigue Severity Scale; R-FSS=Rasch-build Fatigue Severity Scale; MFI=Multidimensional Fatigue Inventory; VAS-F=Visual Analogue Scale for fatigue; NA=not applicable.

Respiratory dysfunction can also contribute to the perceived fatigue. This results from the decreased physical endurance on the one hand and on the other hand from nocturnal hypoventilation causing sleep disturbances.[58]

In multiple sclerosis, cerebrovascular disease, and in spinocerebellar ataxia, depression has a substantial influence on the severity of fatigue.[46 50 51 53 59] This seems not to be a major factor in Pompe disease given the only slightly lower perceived cognitive well-being and low prevalence of depression.[37 38]

Fatigue in Pompe disease can be managed in the following way: I) rule out other possible causes of fatigue; II) evaluate the pulmonary function both in sitting and supine position and if necessary start mechanical ventilator support; III) screen for sleep disorders, including sleep-disordered breathing and install treatment when needed; and IV) optimize supportive care [**Chapter 4.1**]. The provided care involves several medical specialties, which emphasizes the importance of a multidisciplinary care program. The effect of ERT on fatigue is discussed in the subsequent paragraph. The recently conducted training study evaluated the effect of physical exercise on experienced fatigue by adult patients with Pompe disease receiving ERT. The results are shortly awaited. In case training does have a positive effect on fatigue, just as observed in GBS patients,[45 60] this should be incorporated into the care program for fatigued patients with Pompe disease.

Main findings on fatigue in adults with Pompe disease
- Two-thirds of Pompe patients suffer from severe fatigue, which is comparable to the prevalence of severe fatigue in other neuromuscular disorders and in multiple sclerosis.

Recommendations for clinical practice
- It is important to realize that fatigue contributes to the perceived disability in patients with Pompe disease. Complaints of fatigue should be taken seriously and managed adequately by a multidisciplinary team.

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ENZYME REPLACEMENT THERAPY

Effects of enzyme replacement therapy

The timely initiatives taken to start a prospective study in children and adults with Pompe disease and to initiate the IPA/Erasmus MC Pompe Survey have led to the unique situation that most treated patients were prospectively followed before they received ERT. It gave us the opportunity to compare the course of disease before and after the start of ERT. In this way we could demonstrate that ERT positively alters the natural course of Pompe disease in adults reflected by improved muscle strength and stabilized pulmonary function in upright position [**Chapter 3.1**]. Other studies also found great-

est effects on muscle strength and function (**Table III**),[10 29 61-69] with the highest level of evidence coming from the only performed randomized-controlled trial on the effects of ERT (LOTS).[29]

Improvements in muscle strength reported by our group are more substantial than those reported by others,[29 61 63 66-69] as over 45% of our patients were good responders, 46% moderate responders, and only 9% did not benefit from ERT (**Figure 1**). The better response in our study could have several reasons. One is that we were able to compare the disease progression before and after treatment and rated not only improvement but also slowdown of progression as a positive response. Another reason is that we could not correct for the placebo effect as was done in the randomized controlled clinical trial (LOTS). Other reasons for the difference could be that we included patients across the clinical spectrum, that we tested more muscle groups than others, and that we systematically followed a large group of patients for a long period (>500 measurements) as compared to other studies.[29 63 66 68 69] In adult patients, the effect of ERT on muscle strength levels off with longer treatment duration.[10 29 66 69] This trend was seen in LOTS, as patients obtained the largest improvement in walking distance within the first 6 months of treatment. Also our finding that the MRC sumscore did not develop linearly over a 36 months treatment period [**Chapter 3.3**] indicates that the muscle strength tends to stabilize after an initial increase following ERT. This all might indicate that the maximal regenerative capacity of the muscles is reached after a certain time of treatment. With respect to this matter, the awaited long-term treatment data are of great interest.

It was surprising that women would benefit more from ERT than men with respect to muscle strength. In retrospect, the subgroup analysis in LOTS also suggested a better response in women than in men.[29] There is no real clue as to what factors might cause the difference; a difference in lean body mass, muscle fiber size and type (females have smaller muscle fibers, and therefore a higher ratio of muscle-fibre surface to muscle-fibre volume, favoring the mannose 6-phosphate-receptor mediated uptake of ERT), activity patterns, and sex hormone status are amongst the possibilities.

We were the first to report on the course of pulmonary function in supine position during ERT. Though we found an ongoing decline in FVC_{supine} at group level, it is hopeful that the rate of decline after start of ERT was about twice as slow as in the period prior to start of ERT. Moreover, in 61% of the patients the decline in FVC_{supine} was slowed down or halted by ERT (**Figure 1**). The group of non-responders for FVC_{supine} was characterized by older age and more advanced disease status at start of ERT. These findings likely imply that loss of diaphragmatic function is more difficult to restore than the loss of skeletal muscle function, especially in the older and more severely affected patients. A similar observation was made in GAA knock-out mice: ERT restored skeletal muscle better than the diaphragm.[70] The difference might be related to an effect of age on diaphragmatic

function. For instance, it was published that diaphragms of old rats compared to young rats have a slower contraction and relaxation speed due to changes in the Ca2+-ATPase activity of the sarcoplasmic reticulum and changes in the major myosin heavy-chain isoform composition.[71] Whatever the reason, timely start of ERT seems especially important for the preservation of pulmonary function. A currently conducted pilot-study uses cine MRI for investigating respiratory muscle movement in adult patients. The method provides insight in the extent of diaphragm dysfunction and may be a useful tool for monitoring treatment response in Pompe disease (S.C.A. Wens et al, submitted).

As postulated before, fatigue clearly impairs Pompe patients in their everyday lives. Thus, it is an encouraging finding that ERT reduces the level of experienced fatigue on a group level [**Chapter 4.3**]. Also here, women and patients with a shorter disease duration may experience a more distinctive improvement (results did not reach statistical significance). The recently conducted training study performed in our center also assessed the additional effect of exercise on self-experienced fatigue in adult patients receiving ERT. The results are shortly awaited.

On the basis of the current clinical experience with ERT, it seems that all patients with a confirmed diagnosis of Pompe disease, who are clearly symptomatic should best start with ERT unless there are counter indications such as co-morbidities. It seems justified to postpone ERT, if patients have only a raised serum creatine-kinase or very limited limb-girdle muscular weakness without pulmonary involvement. In those cases the best strategy for determining the start of ERT is regular monitoring using standardized protocols. In this way, the best guarantee is obtained that any change in the patients' clinical condition will be noticed. Treatment with alglucosidase alfa every other week can be experienced by the patient as a burden and is very expensive. Thus, the decision to

Main findings on enzyme replacement therapy (ERT) in adults with Pompe disease
- ERT positively alters the natural course of Pompe disease in affected adults.
- ERT improves muscle strength and halts further decline in pulmonary function (upright position) in the majority of patients.
- Despite ERT, progression of pulmonary dysfunction in supine position continues in 39% of the patients.
- ERT reduces the burden of fatigue.
- Female gender, younger age, and better clinical status seem to be predictive factors for favorable disease outcome.

Recommendations for clinical practice
- The expert opinion is to start ERT in clearly symptomatic patients with a confirmed diagnosis of Pompe disease, especially when pulmonary function is affected. In asymptomatic patients or patients with very mild limb-girdle weakness and a stable disease course, it seems justified to apply a watch and wait policy.

Table III Overview of clinical studies on the effects of enzyme replacement therapy in childhood and adult Pompe disease [a,b]

	No. of patients	ERT duration (months)	Age at start ERT (years)	Outcome measures [c]			Fatigue/ Handicap/ QoL	Survival
				Muscle strength	Muscle function	Pulmonary function		
Winkel et al. 2004 [133]	3 [d,e]	36	11,16,32	MRC: (n=2)↑, (n=1)↓; HHD: (n=2)↑, (n=1)≈	GMFM: (n=1)≈, (n=1)↓; PEDi: (n=3)↑	VC: (n=2)↑, (n=1)≈	NR	NR
Rossi et al. 2007 [67]	3 [f]	5,17,30	3,2,19	MRC: (n=1)≈, (n=1)↓	GMFM: (n=2)↑; WGM: (n=2)≈; PEDi: (n=1)≈, (n=1)↑	(n=1) less infections	NR	NR
van Capelle et al. 2008 [134]	3 [g]	96 (initial 36 described by Winkel et al. 2004)	11,16,32	HHD: (n=2)↑, (n=1)≈	GMFM: (n=3)≈	VC: (n=3)≈	RHS: (n=3)↑; FSS: (n=3)↑; SF-36: (n=3)↑	NR
Strothotte et al. 2009 [68]	44	12	49 (21 – 69)	MRC: overall ≈	Arm function test/ Timed tests: overall ↑/≈; WGM: overall ≈	FVC: overall ≈	SF-36: overall ≈	NR
Angelini et al. 2009 [135]	11	3 – 18	42 (22 – 66)	NR	6WT: overall ↑/≈	FVC: overall ↑/≈	SF-36: (n=3)↑	NR
Merk et al. 2009	4	6	39,41,61,68	NR	6WT: (n=1)↑, (n=1)≈	FEV_1: (n=2)↑, (n=2)≈; MIP: (n=3)↑	SF-36: (n=3)↑, (n=1)≈	NR
van der Ploeg et al. 2010 [39,h]	90 (60 treatment arm) [h]	18	alglucosidase 45 (15 – 70); placebo 43 (10 – 68)	QMT: no significant difference between groups	6MWT: difference in favor of alglucosidase alfa	$FVC_{sitting}$, MEP: difference in favor of alglucosidase MIP: no significant difference between groups	SF-36 (PCS): no significant difference between groups	NR
Bembi et al. 2010 [10]	24	>36	juveniles 12 (SD 3.3) adults 48 (SD 10.7)	NR	6WT: (n=24) overall ↑; WGM: (n=24) overall ↑	VC: (n=24) overall ↑; FEV_1: (n=24) overall ≈	NR	NR
Ravaglia et al. 2010 [136]	11	>24	54 (SD 11.2)	HHD: (n=11) overall ≈	6WT: (n=11) overall ↑; Walton score: (n=11) ≈	FVC: (n=11) overall ↑	NR	NR
Kobayashi et al. 2010 [62]	4	>12	17,28,36,44	MRC: (n=4)↑	WGM: (n=4)≈↑	NR	NR	NR
van Capelle et al. 2010 [137]	5	36	11 (5 – 15)	MRC/HHD: (n=5)↑	6WT: (n=1)↑, (n=4)≈; Timed tests: (n=5)↑; QMFT: (n=5)↑	$FVC_{sitting}$: (n=2)↑, (n=3)≈; FVC_{supine}: (n=3)↑, (n=2)≈	NR	NR
Yang et al. 2011 [138]	13	5 – 59	26 (14 – 45)	NR	WGM: (n=13) overall ≈	FVC: after 12 months ERT (n=5)↑/≈ after 48 months ERT (n=2)↑	NR	NR
Orlikowski et al. 2011 [63]	5	12	28,40,48,61,62	QMT: (n=5) ≈	MFM: (n=3)↑, (n=2)≈; Arm/ leg function test/ WGM: (n=5) ≈	$VC_{sitting}$: (n=2)↑, (n=3)↓; VC_{supine}: (n=2)↑/≈, (n=1)↓↘; MIP: (n=4)↑, (n=1)↓; MEP: (n=3)↑, (n=2)↓	FSS: (n=4)↑, (n=1)↓	NR

	No. of patients	ERT duration (months)	Age at start ERT (years)	Outcome measures[c]				
				Muscle strength	Muscle function	Pulmonary function	Fatigue/ Handicap/ QoL	Survival
Papadimas et al. 2011[64]	5	6-36	40,40,43,47,73	MRC: (n=2) ↑, (n=2) ↑/≈, (n=1) ≈	NR	$FVC_{sitting}$: (n=2) ↑, (n=3) ↓ FVC_{supine}: (n=2) ↑/≈ FEV_1: (n=2) ↑, (n=2) ≈	FSS: (n=2) ↑, (n=2) ≈	NR
Furusawa et al. 2012[61]	5	24	32,38,44,55,66	MRC: (n=5) ≈	GMFM: (n=5) ↑/≈ Barthel index: (n=5) ≈	(F)VC/ FEV_1: (n=5) ↑/≈	NR	NR
Angelini et al. 2012[139]	74	12 – 54	43 (7 – 72)	NR	6.WT: (n=58) overall ↑ WGM: (n=68) overall ≈	$FVC_{sitting}$: (n=69) overall ≈	NR	NR
Regnery et al. 2012[66]	38	24 (initial 12 described by Strothotte et al. 2009)	51 (23 – 68)	MRC: (n=38) overall ≈	6.WT: (n=38) overall ↑ Arm function test/ WGM/ timed tests: (n=38) overall ≈	$FVC_{sitting}$: (n=38) overall ≈	SF-36: (n=38) overall ≈	
van der Ploeg et al. 2012[69]	55	24 (initial 18 described by van der Ploeg et al. 2010)	45 (15 – 70)	QMT arm: (n=52) overall ↑ QMT leg: (n=53) overall ≈	6.WT: (n=53) overall ≈	$FVC_{sitting}$: (n=53) overall ≈ MIP: (n=53) overall ↑ MEP: (n=53) overall ↑	NR	NR
de Vries / van der Beek et al. 2012[26] **Chapter 3.1**	69	23 (5 – 47)	52 (26 – 76)	MRC: (n=69) overall ↑ HHD: (n=64) overall ↑	QMFT: (n=69) overall ≈/↑	$FVC_{sitting}$: (n=62) overall ≈ FVC_{supine}: (n=54) overall ↓	NR	NR
Güngör et al. 2013[140]	283	48	48 (19 – 81)	NR	NR	NR	NR	(n=204) overall ↑ (HR 0.41)
Güngör et al. 2013[37] **Chapter 4.3**	163	36	50 (24 – 76)	NR	NR	NR	FSS: (n=163) overall ↑	NR

Table adapted from the thesis written by N.A.M.E. van der Beek.

↑ = improvement; ↓ = decline; ≈ = stable or no further changes; No=number; ERT=Enzyme Replacement Therapy; QoL=Quality of Life; NR=not reported; MRC=Medical Research Council; HHD=Hand-Held Dynamometry; QMT=Quantitative Muscle Testing; 6MWT=Six-Minute Walk Test, QMFT=Quick Motor Function Test; PEDI=Pediatric Evaluation of Disability Inventory; WGM=Walton-Gardner-Medwin scale; MFM=Motor Function Measure; (F)VC=(Forced) Vital Capacity; FEV_1=Forced Expiratory Volume in 1 second ; MIP=Maximal Inspiratory Pressure; MEP=Maximal Expiratory Pressure; RHS=Rotterdam Handicap Scale; FSS=Fatigue Severity Scale; SF-36=medical outcomes study 36-item Short-Form health survey; PCS = Physical Component Summary.

[a] Studies with more than 3 patients are mentioned, [b] the study has a observational open-label design and the dosing regimen of enzyme replacement therapy is 20 mg/kg every other week unless otherwise specified, [c] for the studies with more than 5 patients the effect at group level is presented, otherwise the outcome per number (n) of patients is given,[d] rabbit derived recombinant human acid α-glucosidase, [e] dosing regimen initially 10/mg/kg/week, later 20 mg/kg/week, [f] dosing regimen: 1 patient initially 10 mg/kg/2 weeks, gradual increase to 40 mg/kg/2 weeks by week 96, [g] dosing regimen 30-40 mg/kg/2 weeks, [h] randomized placebo-controlled clinical trial.

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start treatment should be made carefully and should be made by a group of specialists in the field (indication committee).

Anti-alglucosidase alfa antibody response

Our findings and those of others show that ERT improves the patient's prospects and slows or halts disease progression in 60-90% of patients (**Table III**). However, with growing experience it is also recognized that not all patients benefit equally from ERT, and that there are marked variations in treatment response. In classic-infantile Pompe disease, part of this variation is clarified by the development of high sustained antibodies titers attenuating treatment efficacy.[72] [73] Also in other lysosomal storage diseases, immune responses to recombinant enzymes are a well-known phenomenon, since these are encountered in treated patients with Gaucher disease,[74] Fabry disease,[75-78] and Mucopolysaccharidosis type I, II and VI (**Table IV**).[79-83] The proportion of patients that develop antibodies varies from 2% to 100%, with the lowest proportions seen in Gaucher disease. The biggest concern is that antibodies may impair the efficacy of the infused protein, but no adverse effects of antibody formation on therapeutic efficacy could be demonstrated in these other lysosomal storage diseases.
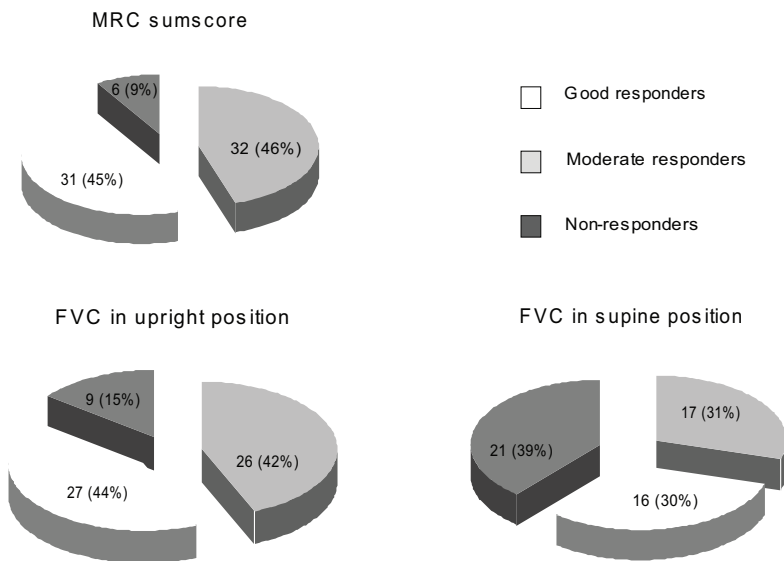
Figure 1 Individual response groups for skeletal muscle strength and pulmonary function
The number and percentage of good responders, moderate responders and non-responders are shown for MRC sumscore, and forced vital capacity (FVC) in upright and supine positions.

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Table IV Recommended doses, infusion-associated reactions, and antibody formation of human recombinant enzymes used to treat lysosomal storage diseases

Disease	Subtype	Recombinant enzyme	Dose and schedule	IARs	Antibody formation
Gaucher disease	Type 1	β-Glucocerebrosidase: imiglucerase (Cerezyme®)	1.6 mg/kg eow	14%	15%
		β-Glucocerebrosidase: velaglucerase alfa (VPRIV®)	1.6 mg/kg eow	52%	2%
Fabry disease	Both classic and later onset	α-Galactosidase A: agalsidase beta (Fabrazyme®)	1.0 mg/kg eow	50-55%	68%
		α-Galactosidase A: agalsidase alfa (Replagal®)	0.2 mg/kg eow	52%	64%
MPS type I	Hurler-Scheie and Scheie syndromes	α-L-Iduronidase: laronidase (Aldurazyme®)	0.58 weekly	32%	97%
MPS type II	Both severe and attenuated	Iduronate-2-sulfatase: idursulfase (Elaprase®)	0.5 weekly	15%	47%
MPS type VI	-	N-Acetylgalactosamine-4-sulfatase: galsulfase (Naglazyme®)	1.0 mg/kg weekly	55%	97-100%
Pompe disease	Classic-infantile	Acid α-glucosidase: alglucosidase alfa (Myozyme®)	20-40 mg/kg eow/weekly	51%	95-100%
	Non-classic	Acid α-glucosidase: alglucosidase alfa (Myozyme®/Lumizyme®)	20 mg/kg eow	5-28%	97-100%

Table adapted from the publication by Desnick and Schuchman, entitled Enzyme Replacement Therapy for Lysosomal Diseases: Lessons from 20 Years of Experience and Remaining Challenges in Annual Review of Genomics and Human Genetics 2012, by permission of Annual Reviews.[141]
*IARs = Infusion-Associated Reactions; eow=every other week; VPRIV=Velaglucerase Alfa for Injection; MPS=Mucopolysaccharidosis*

In adults with Pompe disease, it was anticipated that antibody formation would not play a substantial role in treatment outcome since most of these patients produce a fair amount of acid α-glucosidase themselves. This was corroborated by the findings of LOTS, wherein all 59 treated patients did develop antibodies, but not to such extend that the effect of treatment was annihilated. In one of our adult patients and in three patients reported by Patel *et al.*,[84] high sustained antibody titers however coincided with progressive disease and persisting IARs during ERT [**Chapter 3.2**].[85] By performing pharmacokinetic studies in our patient with a very high antibody titer it was demonstrated that the patient's anti-rhGAA antibodies captured 40% of the administered alglucosidase alfa and inhibited *in vitro* uptake of enzyme and enzyme activity. In a subsequent study, we investigated the potential impact of antibodies on the treatment safety and efficacy in a large group of adult patients [**Chapter 3.3**]. Of the 73 patients, 71 (97%) developed any titer of antibodies against alglucosidase alfa. The mean titer peaked at 6 months after start of ERT to 1:1259 and declined to 1:270 at 36 months of ERT. *In vitro*, high titers of anti-alglucosidase alfa antibodies had neutralizing effects on the activity of alglucosidase alfa in the medium and in the target cells to a similar extent, indicating that the predominant and relevant effect of the antibodies was inhibition of enzymatic activity rather than inhibition of uptake of alglucosidase alfa by the cells (cultured fully GAA deficient fibroblasts).

The findings obtained by *in vitro* studies did not correspond fully with the clinical findings since no statistical significant differences were found in treatment outcome across patients with high (≥1:31,250), intermediate (1:1250 -<1:31,250) and low or no (0 - 1:1250) antibody titers towards alglucosidase alfa. We did find an increased risk for IARs in patients with higher antibody peak titers: 44% of the patients with high titers experienced IARs, 19% of the patients with intermediate titers, and a mere 3% of the patients with a low or no titer. An explanation for the clinical deterioration in the few reported cases with very high antibody titer,[29 84 85] could be that there are some particularly destructive epitope-binding sites (**Figure 2**).[86]

Based on our findings and those of others,[29 84 85] the immune response to alglucosidase alfa has a far lower impact on treatment outcome in adults than in infants with regard to muscle strength and pulmonary function. Thus, in adults there seems no need for the implementation of tolerance inducing therapies such as rituximab combined with methotrexate and intravenous immunoglobulins or omalizumab as has been practiced in classic infantile Pompe disease.[87 88] Patients, who develop low or no antibody titers (<1:1250) within the first 6 months of ERT, have a very low risk of IARs. Thus they can be transferred earlier to home treatment. On the other hand close monitoring of IARs remains important in patients with substantial antibody titers (≥1:1250).
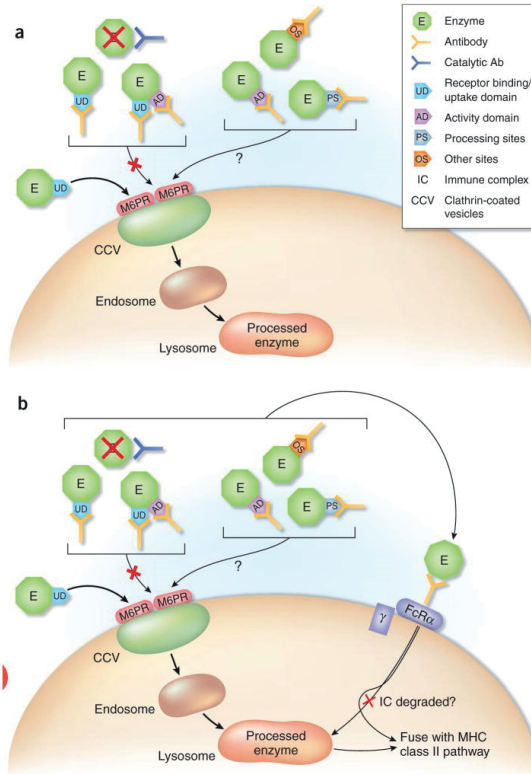
Figure 2 Impact of neutralizing antibody on enzyme uptake and activity in ERT
A There are several steps by which therapeutic enzymes for Pompe disease and other lysosomal storage diseases can influence effectiveness. First, uptake of the enzyme (E) is mannose-6-phosphate (M6P) receptor-mediated, endocytosed by clathrin-coated vesicles (CCV) and fused with endosomes and then with lysosomes, where enzymatic activity breaks down the accumulated substrate. There are several possible outcomes of the binding of lysosomal enzyme–specific antibodies (yellow 'Y'): blockade of enzyme uptake through M6P-receptor by binding to the receptor binding or uptake domain(s) (UD; domains containing exposed M6P); blockade of enzyme uptake by M6P-receptor and suppression of enzymatic activity by binding to epitopes near the receptor binding domain and the enzymatic activity domain (AD; theoretical); blockade of both uptake and activity domains by separate antibodies specific for each site; degradation of the enzyme by catalytic antibody (red 'Y'); reduction of enzymatic activity by targeting the enzymatic domain; prevention of enzyme maturation by targeting the enzyme protease processing sites (PS); and targeting of other sites (OS) of the enzyme, resulting in conformational or trafficking changes. **B** Binding of antibodies to enzyme may redirect the enzyme to FcR-expressing cells, such as macrophages and B cells. Enzyme–antibody complexes internalized through FcRs may prevent proper translocation of functional enzymes to the lysosome. Binding of antibody to other domains of the enzyme may change pharmacokinetics or redirect the enzyme to FcR-expressing cells. IC, immune complex. This figure originates from Wang et. al. Neutralizing antibodies to therapeutic enzymes: considerations for testing, prevention and treatment. *Nature Biotechnology* 2008, by permission of the Nature Publishing Group.

Enzyme replacement therapy during pregnancy and lactation

In other lysosomal storage diseases the experience with continuation of ERT during pregnancy, though in much lower dosages than in Pompe disease, did not suggest major problems for the unborn child of the treated mother. Based on this experience together with the reporting of normal fetal development in mice and rabbits treated with alglucosidase alfa, [89-93] we assumed that the continuation of ERT during pregnancy would not pose a substantial risk for the mother and her child. Our first patient, a 40-year old woman, had been receiving ERT for 17 months before she became pregnant. She had moderate limb-girdle weakness and used non-invasive nightly ventilation. Her FVC in seated position was 44% of predicted and dropped to 31% predicted in the supine position. Close monitoring of the pregnancy showed that there were no maternal complications and the child developed normally [**Chapter 3.4**].[94] Very low levels of alglucosidase alfa were detected in the breast milk on the day of infusion. Within 24 hours, the activity level in the breast milk was back to the pre-infusion level. Therefore, there seems little rationale to entirely refrain from breastfeeding while mothers are on ERT. To be on the safe site, we advise our patients to discontinue breastfeeding on the day of the infusion. Our experience with a second pregnancy case (unpublished results) and a pregnancy case reported by Zagnoli *et al.* [95] also indicated that ERT can be safely administered during pregnancy. Since it concerns only single case-reports, we underline that careful monitoring of each pregnancy remains important.

Main findings on antibodies and continuation of ERT during pregnancy and lactation
- The majority of ERT treated adult Pompe patients develop antibodies against alglucosidase alfa.
- Antibodies against alglucosidase alfa are associated with an increased risk for infusion-associated reactions
- In the majority of patients with high titer antibodies, no adverse effects on clinical outcome could be demonstrated.
- Continuation of ERT during pregnancy and lactation seems safe for mother and child.

Recommendations for clinical practice
- Immune surveillance in ERT treated children and adults with Pompe disease remains important for investigating whether anti-alglucosidsae alfa antibodies have adverse effects on long-term treatment outcome.
- Early implementation of home treatment is justified in patients who do not develop a substantial antibody response (≤1:1250) in the first 6 months of treatment.
- There seems no need for the discontinuation of ERT in pregnant and breastfeeding women with Pompe disease.

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FUTURE PERSPECTIVES

The findings described in this thesis demonstrate that the introduction of ERT has altered the lives of Pompe patients in a positive way. They also demonstrate that the implementation of a new treatment involves new challenges, such as the fact that we are still poorly able to explain the observed variability in response. By deciphering the mechanisms that hamper treatment efficacy we might be able to improve the therapeutic efficacy of ERT. At the same time, scientific efforts are ongoing to develop the ultimate curative treatment for this disease.

Improving the efficacy of enzyme replacement therapy

The following approaches can or may increase the therapeutic efficacy of ERT: I) shortening the delay in diagnosis; II) establishing predictive factors for treatment outcome; III) glycoengineering of rhGAA; IV) peptide modification of rhGAA; V) dose augmentation; and VI) chaperone therapy as an add-on to ERT.

As mentioned earlier, there is much to be gained by *shortening the diagnostic delay*, because of the presence of substantial disability in the majority of the patients at time of diagnosis that could have been prevented by a timely diagnosis and subsequent start of ERT.[14] Shortening of the diagnostic delay can be accomplished by further increasing the disease awareness. In essence the best way to prevent a diagnostic delay is newborn screening. However, since the majority of patients become symptomatic later in life, a drawback to making a diagnosis just after birth is that it creates a lot of 'patients-in-waiting'.[96] The feasibility of newborn screening is currently subject of study.[97-100] The pros and cons are being carefully considered, before a final decision will be made whether to add Pompe disease to the newborn screening program.

Studying *predictive factors* for treatment outcome is a challenge in Pompe disease, as statistical power is often limited by the small sample sizes inherent to rare disorders. For this reason, it is essential to collect reliable data in the context of large international collaborative studies or international patient registries. In this way enough power is generated for applying a full multivariate model and developing a prognostic model for individual treatment outcome. Such a model could assist in clinical decision making for the individual patient and in developing treatment guidelines. Real progress is awaited this year, as the topic of the upcoming ENMC meeting this September is about the formation of a European network to develop treatment guidelines and prognostic models for non-classic Pompe disease.

Approach III, IV and V are methods for enhancing the uptake of alglucosidase alfa in the most affected tissues. As shown in a GAA knock-out mice model, most of the recombinant enzyme is captured by the liver and the spleen, and only a minor fraction is taken

up by the heart and the skeletal muscle tissue.[101] By enhancing the uptake by skeletal muscle tissue, the therapeutic efficacy could be improved.

D*ose augmentation* is the easiest way to ameliorate the therapeutic effect of ERT. In classic infantile Pompe disease there is increasing evidence that a weekly dose of 40 mg/week improves ventilator-free survival and motor outcome compared to the currently recommended dose of 20 mg/kg every other week (eow).[102 103] Based on theoretical pharmacokinetic modeling based, it is supposed that weekly dosing, and not per se higher dosing contributes most to the better clinical outcome.[102] This is supported by the findings of the only conducted (open) study in classic-infantile Pompe disease comparing a dosing schedule of 20 mg/kg eow to 40 mg/kg eow.[104] No differences with regard to safety and therapeutic efficacy over a 3 years of treatment were observed. In children and adults there is only one single case report about the improvement of ptosis in a 17-year old adolescent with Pompe disease after increasing the dose from 20 mg/kg to 40 mg/kg, every other week.[105] It would be of interest to systematically study the effect of dose augmentations in childhood and adult Pompe disease. With higher dosing one should realize that the costs of treatment increase as well as the risk for antibody response and IARs.[106]

Glycoengineering, explained below is an alternative way to enhance the uptake of recombinant human acid α-glucosidase by muscle tissue. The uptake of alglucosidase alfa is mannose 6-phosphate (M6P)-receptor mediated, however the currently produced recombinant human alglucosidase alfa has low numbers of M6P groups. *In vitro* studies have shown that the uptake of acid α-glucosidase by cultured fibroblasts and muscle cells improves when the number of M6P-moieties increases. Enrichment of the enzyme with M6P-moieties can either be accomplished by genetic plus enzymatic engineering (hypermannose-6-phosphorylated enzyme (HP-GAA)) or by the chemical conjugation of synthetic oligosaccharides bearing M6P residues (neo-rhGAA/oxime-neo-rhGAA).[107-110] The intended gain would be that the treatment efficacy per milligram infused recombinant enzyme increases. The method of carbohydrate modification bears the risk of increasing the immunogenicy of the recombinant enzyme and it is therefore important to compare the safety profile of *glycoengineered forms of* recombinant human acid α-glucosidase with the currently used alglucosidase alfa. Moreover, as the production process of alglucosidase is already quite costly, the question is whether additional manufacturing steps will not lead to even higher costs.

The development of the *peptide modified* alternative form of ERT, BMN 701 (BioMarin) is ongoing. The start of the phase 2/3 study (ClinicalTrials.gov: NCT01924845) is planned for this year. BMN 701 is a fusion of insulin growth factor 2 (IGF-2) and GAA, and takes advantage of the fact that the peptide IGF-2 also binds to the M6P receptor. Therefore, BMN 701 can be taken up into the muscle cells via the M6P receptor. The results of the

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phase 1/2 study with a follow-up of 24 months in 16 patients showed improvements in the distance walked during the 6MWT and in FVC in upright seated position comparable to the findings of LOTS. Effects on MIP and MEP were more pronounced.

Another recent development is the application of *chaperone therapy* as an add-on to ERT. Chaperone therapy is based on the principle that missense mutations can lead to improperly folded or unstable proteins. Chaperones are small molecules that selectively bind to the enzyme thereby stabilizing its structure and improving intracellular trafficking to the lysosomes.[111] A disadvantage of this therapy is that it only fits certain missense mutations and will certainly not have an effect on null-mutations, frameshift mutations, and splice site effects.[112]

The first clinical application of *chaperone therapy* as a monotherapy for Pompe disease has been unsuccessful, because of the occurrence of severe side effects in two adult Pompe patients in a phase 2 study. The patients developed progressive muscle weakness and a marked rise in serum creatinine-kinases. This adverse effect is best explained by inadequate dosing, whereby persistent binding of the chaperone to endogenous acid α-glucosidase might block the catalytic site permanently and increase the enzyme deficiency. The inhibitory effect of the administered chaperone on intestinal maltase activity was not mentioned to play a role, but might have contributed to the general malaise of the patients receiving *chaperone therapy* as monotherapy in Pompe disease. To overcome this problem, new chaperones have recently been identified (e.g. N-acetylcysteine) that do not bind to the active-site of the enzyme and therefore do not counteract enzyme function.[113] [114]

Subsequent research has focused on whether *chaperones* might have a synergistic effect as adjuvant to ERT with alglucosidase alfa. *In vitro* studies as well as studies carried out in GAA knock-out mice have shown that chaperone therapy combined with ERT increases the lysosomal α-glucosidase activity and the lysosomal glycogen clearance.[115] [116] The results of clinical studies performed in patients with Pompe disease are awaited.

Future therapies

Since the effectiveness of ERT depends on life-long intravenous enzyme administrations, imposing a burden to the patients, there remains a demand for curative, even more effective, or less invasive intervention.

Gene therapy uses the principle of transferring an intact copy of acid α-glucosidase cDNA to the cells of a Pompe patient, thereby generating a constant production of the enzyme. Previous *in vitro* and *in vivo* studies are supportive for the practicability of gene therapy in Pompe disease. For delivery of the GAA gene construct, retroviral and adenoviral vectors expressing human acid α-glucosidase were studied in *in vitro* experiments. This resulted in the production of catalytically active acid α-glucosidase with ac-

tual reduction of the lysosomal glycogen content.[117-121] *In vivo* studies performed in GAA knockout mice showed that intramuscular or intramyocardial injections of the vectors led to acid α-glucosidase expression in only the directly adjacent areas, but not in distant muscle groups[118 122 123] Transduction of hepatic cells with adeno-associated viral vectors carrying the GAA cDNA construct was successful and resulted in sufficient production of acid α-glucosidase and secretion in the circulation with subsequent uptake in the skeletal muscles. Unfortunately, the previously used strategies induced fierce immune responses hampering the long-term efficacy of this treatment.[124-126]

The fact that the diaphragm is rather unresponsive to ERT has led to the initiation of a Phase I/II trial wherein an acid α-glucosidase expression construct based on adeno-associated virus technology (rAAV1-CMV-GAA) is directly injected into the diaphragm. The results of this study are awaited in 2016 (ClinicalTrials.gov: NTC00976352).

Hematopoietic stem-cell transplantation (HSCT) replaces lineages of blood stem-cell derived enzyme deficient cells by proficient cells with a chance that the enzyme produced by the donor cells spreads to other cells and tissues. Theoretically, the transplanted cells can serve as permanent source of newly synthesized acid α-glucosidase, which can be taken up by the enzyme deficient skeletal muscle cells.[127] So far, the clinical application of *HSCT* for the treatment of lysososmal storage disorders is limited to a subset of patients with mucopolysaccharidosis type 1 (Hurler syndrome), Krabbe disease, and metachromatic leukodystrophy. *HSCT* has been unsuccessful in the few patients with Pompe disease that underwent this treatment.[128 129] Several recent studies indicate that *HSCT* using Lentiviral vectors may become a promising approach in the future.[130-132] The first results of a clinical trial in another lysosomal storage disorder than Pompe disease have been published.

Final remarks

Important steps forward have been made, and further steps can be made by combining specialized multidisciplinary care with clinical and fundamental research as has materialized in our Center of Lysosomal and Metabolic Diseases at Erasmus MC. As explicated in this thesis, the introduction of enzyme replacement therapy has had a great impact

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Future directions
- Nationwide and international collaboration is needed to estimate the long-term effects of ERT and to develop prognostic models by which the treatment for individual patients can be optimized and define start and stop criteria further.
- Improvement of the efficacy of ERT starts with timely diagnosis and timely treatment with an optimal dosing regimen. New products and new approaches can be of further help.
- The development of gene therapy based strategies has to be pursued with great energy in order to obtain curative intervention in the near future.

on the prospects for patients with Pompe disease. But, there is undoubtedly room for further improvement. Currently, European collaboration is ongoing aiming at identifying predictive factors for treatment response and developing uniform treatment guidelines. Clinical studies on the efficacy of ERT with new products will start soon, and the results are eagerly awaited. Meanwhile, the development of gene therapy is actively pursued. These combined efforts will hopefully gradually change the life of Pompe patients for the better.

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CHAPTER 6

Summary
Samenvatting

SUMMARY

Pompe disease is an autosomal recessive metabolic myopathy caused by a deficiency of the lysosomal enzyme acid α-glucosidase due to mutations in the GAA gene. Infants with a total deficiency accumulate glycogen in almost all their tissues, but primarily manifest skeletal and cardiac muscle pathology. Children and adults with a partial deficiency present the first symptoms later in life, and the pathologic changes emerge mainly in skeletal muscle tissue. Without enzyme replacement therapy (ERT), affected infants have a life expectancy of maximally 2 years. The majority of diagnosed adults eventually becomes wheelchair-bound and dependent on mechanical ventilation when they are not treated. This thesis focuses on adults with Pompe disease.

Enzyme replacement therapy (ERT) using alglucosidase alfa (Myozyme®) is the first disease-specific treatment for an inherited neuromuscular disorder, which makes the situation for Pompe disease rather unique. Registration of ERT in 2006 was predominantly based on the positive results obtained from clinical trials performed in infants with classic infantile Pompe disease. Information on the effects and safety of the use of alglucosidase alfa in children and adults with Pompe disease was limited at start of the research described in this thesis.

For that reason, the Dutch nationwide prospective observational study on the natural course and the effects of ERT in children and adults with Pompe disease started in 2004 at Erasmus MC. In addition, patient-reported information on handicap, quality of life and fatigue was gathered via the international IPA(International Pompe Association)/Erasmus MC Pompe Survey.

The scientific work presented in this thesis is focused on the clinical manifestations, natural history, and the effects and safety of treatment with alglucosidase alfa in adult Pompe disease. The aims were to investigate: 1) the clinical features and natural course; 2) the effects of ERT and prognostic factors for treatment response; 3) the impact of anti-alglucosidase alfa antibody formation on clinical outcome in patients treated with ERT; and 4) whether the continuation of ERT was safe during pregnancy and lactation. The final aim was to describe the burden of fatigue in neuromuscular diseases, with a focus on Pompe disease and Guillain-Barré syndrome, and to evaluate the effect of ERT on fatigue in adults with Pompe disease.

Chapter 1 – the General Introduction – provides information on the clinical spectrum and manifestations, diagnosis, pathogenesis and treatment of Pompe disease. The end of this chapter accounts for the scope and the aims of the thesis.

In **Chapter 2.1** the clinical manifestations, natural course and predictors for disease progression are described in a cohort of 94 patients with a median age of 51 years (range 25 to 75 years). Data were prospectively collected at Erasmus MC between October 2004

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and August 2009 in the context of the previously mentioned Dutch nationwide study. Skeletal muscle weakness was characteristically distributed in the limb-girdle pattern. Less well-known features of the disease were far more common than previously thought, as illustrated by the finding that ptosis occurred in 23%, bulbar weakness in 28%, and scapular winging in 33% of the patients. During the study, skeletal muscle strength declined significantly with a mean of -1.3 percentage points per year (pp/y) for manual muscle testing and -2.6 pp/y for hand-held dynamometry (both P <0.001). On average, forced vital capacity in supine position deteriorated by -1.3 pp/y (P =0.02). Ten percent of patients declined unexpectedly fast. Longer disease duration and reduced pulmonary function were predictors of a more rapidly decline of skeletal muscle strength. **Chapter 2.2** encompasses the first international study reporting on survival in adult Pompe disease. Results were calculated based on follow-up data with a mean of 3.5 years in a total of 268 untreated adult patients participating in the IPA/Erasmus MC Pompe Survey. By comparing the number of deaths to the expected number of deaths in the Dutch general population, it was shown that the mortality rate was higher in patients with Pompe disease. The estimated 5-year survival rate from diagnosis was 95%. The 10, 20, and 30 year survival rates were appraised at 83, 65, and 40%. Predictors of a higher mortality rate were wheelchair use, mechanical ventilation and a higher level of disability as measured with the Rotterdam-Handicap Scale.

   Chapter 3 focusses on the effects and safety of ERT in adult patients with Pompe disease. In **Chapter 3.1** the effects of ERT as observed in the Dutch nationwide prospective study are described and contrasted to the natural disease course. In 69 patients with a median age of 52 years (range 26 to 76 years) muscle strength increased on average with 1.4 pp/y for manual muscle testing and with 4.0 pp/y for hand-held dynamometry over a median treatment duration of 23 months (both P <0.001). Pulmonary function remained stable when measured in upright position, but declined in supine position (-1.1 pp/y; P =0.03). As compared to the natural course, ERT had a beneficial effect on muscle strength (manual muscle testing 3.3 pp/y; hand-held dynamometry 7.9 pp/y; both P <0.001). A favorable prognostic factor for treatment related outcome with regard to muscle strength was female gender. Favorable prognostic factors for a better treatment outcome with regard to pulmonary function (supine) were younger age, less severe limb-girdle muscle weakness and better pulmonary status at start of ERT. **Chapter 3.2** reports on an adult patient with Pompe disease, suffering from ongoing disease progression and continued infusion-associated reactions during treatment with alglucosidase alfa, who developed a high sustained antibody response towards the administered enzyme. It was calculated that the concentration of antibodies in the patient's blood was so high that they would capture approximately 40% of the administered alglucosidase alfa. When tested *in vitro*, the antibodies substantially inhibited the uptake of alglucosidase alfa by cultured fibro-

blasts. These findings are indicative of neutralizing effects of antibodies on therapeutic efficacy. In **Chapter 3.3** it is demonstrated that 71 of the 73 (97%) adult patients develop antibodies to alglucosidase alfa. The mean titer peaked at 6 months to 1:1259 declining to 1:270 after 36 months of ERT. Individual titers and titer courses varied, but in the majority (93%) of patients titers decreased or stabilized with continued enzyme replacement therapy. *In vitro* studies demonstrated that the higher the antibody titer, the more the activity of alglucosidase alfa was inhibited. The proved adverse *in vitro* effect was not reflected in the *in vivo* findings, as no clear adverse effect of high antibody titers could be demonstrated on clinical outcome. We did find that the risk for infusion-associated reactions increased with higher peak titers (P =0.001). In **Chapter 3.4** we describe our experience with a 40-year-old woman with Pompe disease, who continued receiving ERT during pregnancy and lactation. A healthy boy was born at a gestational age of 37 weeks and 5 days by elective Cesarean section. There were no maternal complications and the child developed normally. Alglucosidase alfa was secreted into the breast milk at very low levels during the infusion, but 24 hours after start of the infusion the enzyme activity in the breast milk was back to the pre-infusion level. Based upon these findings, the continuation of treatment with alglucosidase alfa during pregnancy and lactation seems safe for both mother and child.

Chapter 4 addresses excessive fatigue, which is a frequently encountered complaint in patients with a neuromuscular disease. In **Chapter 4.1** the pathophysiological mechanisms of fatigue and its burden are reviewed with a focus on Pompe disease and Guillain-Barré syndrome. Prevalence rates of fatigue in neuromuscular disorders compare to the high levels of experienced fatigue reported in multiple sclerosis. Fatigue can be subdivided into experienced fatigue and physiological fatigue, physiological fatigue subsequently in central and peripheral fatigue. In neuromuscular disorders, peripheral fatigue is an important contributor. Nevertheless, in reaction to the pathology in the peripheral nervous system, fatigue of central origin can also be an important protective mechanism restricting further damage (central governor model). Severity of fatigue seems to be related to disease severity, possibly with the exception of fatigue occurring after a monophasic disorder like Guillain–Barré syndrome. Fatigue in neuromuscular disease can be managed by symptomatic treatment of the underlying disease, exercise and cognitive behavioral therapy. In **Chapter 4.2** we present a case of Pompe disease with an uncommon clinical presentation characterized by severe fatigue and myalgia prior to the onset of limb-girdle weakness. Remarkably, the muscle biopsy demonstrated selective involvement of type 1 muscle fibers. It is hypothesized that this finding might contribute to the uncommon clinical presentation of this patient. In **Chapter 4.3** the effects of ERT on experienced fatigue in 163 patients participating in the IPA/Erasmus MC Pompe Survey are reported. The follow-up included a median period of 4 years before start of ERT

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and of 3 years during ERT. Fatigue severity levels measured with the fatigue severity scale (FSS) remained stable at around 5.3 points before start of ERT. During ERT, a reduction in the average experienced fatigue was measured, as scores improved significantly by 0.13 per year (P <0.001).

Chapter 5 – the General Discussion – outlines the most important findings, compares them to relevant medical literature, and translates them into recommendations for clinical practice. Also potential methods to innovate ERT, new treatment strategies and future research are discussed.

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SAMENVATTING

De ziekte van Pompe is een autosomaal recessieve metabole spierziekte, die ontstaat door een deficiëntie van het lysosomale enzym zure α-glucosidase, welke wordt veroorzaakt door mutaties in het GAA gen. Patiënten met de klassiek-infantiele vorm van de ziekte zijn volledig deficiënt voor het enzym. Dit leidt tot snelle stapeling van glycogeen in vrijwel alle lichaamsweefsels, met de meest uitgesproken pathologische veranderingen in dwarsgestreept spierweefsel en in het hart. Bij kinderen en volwassenen met een gedeeltelijke deficiëntie openbaart de ziekte zich op latere leeftijd, en de pathologische veranderingen treden met name op in de skeletspieren. Voordat behandeling beschikbaar kwam, hadden baby's met de klassiek-infantiele vorm een maximale levensverwachting van twee jaar. De meerderheid van de volwassen patiënten wordt uiteindelijk rolstoel- en beademingsafhankelijk. Dit proefschrift richt zich op volwassen patiënten met de ziekte van Pompe.

Enzymvervangingstherapie (ERT) met alglucosidase alfa (Myozyme®) is de eerste ziekte-specifieke behandeling voor een erfelijke neuromusculaire ziekte. De registratie van het medicijn is tot stand gekomen in 2006 op basis van de positieve ervaringen met de behandeling met ERT van klassiek-infantiele patiënten. Gegevens over de behandeleffecten en de veiligheid van ERT bij kinderen en volwassenen met de ziekte van Pompe waren op het moment van registratie en ten tijde van de start van het onderzoek beschreven in dit proefschrift nog beperkt voorhanden.

Om hier meer gegevens over te verkrijgen is in 2004 de Nederlandse prospectieve observationele studie naar het natuurlijke ziektebeloop en de behandeleffecten van ERT in kinderen en volwassenen met de ziekte van Pompe van start gegaan in het Erasmus MC. Daarnaast werden patiënt-gerapporteerde uitkomsten met betrekking tot handicap, kwaliteit van leven en vermoeidheid verzameld in het kader van het internationale IPA (International Pompe Association)/Erasmus MC Pompe vragenlijst onderzoek.

Het onderzoek in dit proefschrift heeft betrekking op de klinische presentatie, het ziektebeloop, en de effecten en veiligheid van ERT bij volwassenen met de ziekte van Pompe. De doelstellingen van het onderzoek waren: 1) het uitgebreid beschrijven van het klinisch beeld en het ziektebeloop van de ziekte van Pompe; 2) het bepalen van de effecten van ERT en prognostische factoren voor het behandeleffect; 3) het effect van antilichaamvorming op het behandeleffect van ERT onderzoeken; en 4) de veiligheid van ERT onderzoeken tijdens zwangerschap en lactatie. Als laatste hebben we ernstige vermoeidheid bij patiënten met neuromusculaire ziekten beschreven, geïllustreerd aan de hand van vermoeidheid bij de ziekte van Pompe en het Guillain-Barré syndroom. Daarnaast hebben we gekeken naar het effect van ERT op vermoeidheid bij volwassenen met de ziekte van Pompe.

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In **Hoofdstuk 1** – de algemene inleiding – wordt achtergrondinformatie gegeven over het klinisch beeld, ziektebeloop, diagnose, pathogenese en behandeling van de ziekte van Pompe. Op het einde worden de doelstellingen van het onderzoek uiteengezet.

Hoofdstuk 2.1 geeft een uitgebreide beschrijving van het klinisch spectrum en het natuurlijke ziektebeloop van 94 patiënten met een mediane leeftijd van 51 jaar (range 25 tot 75 jaar). Tussen oktober 2004 en augustus 2009 zijn de gegevens verzameld in het kader van de eerder genoemde nationale prospectieve studie uitgevoerd door het Erasmus MC. We vonden dat de spierzwakte volgens het limb-girdle patroon was verdeeld. Minder bekende uitingen van de ziekte kwamen veel vaker voor dan op voorhand gedacht. Ptosis werd in 23%, bulbaire zwakte in 28%, en scalpula alata in 33% van de patiënten gezien. Gedurende de studie verslechterde de spierkracht met -1.3 percentagepunten per jaar (pp/jaar) voor de handmatige geteste spierkracht en met -2.6 pp/jaar voor spierkracht gemeten middels hand-held dynamometrie (beide *P* <0.001). De gemiddelde achteruitgang van de longfunctie in liggende houding was -1.3 pp/jaar (*P* =0.02). Tien procent van de patiënten vertoonde een onverwacht snelle achteruitgang. Een langere ziekteduur en een slechtere longfunctie bij aanvang van de studie waren voorspellers van een snellere achteruitgang in spierkracht. **Hoofdstuk 2.2** betreft de eerste studie naar de levensverwachting van volwassenen met de ziekte van Pompe. De resultaten zijn berekend over een gemiddelde follow-up van drieënhalf jaar van in totaal 268 patiënten die deelnamen aan het IPA/Erasmus MC onderzoek. Door het aantal overleden patiënten te vergelijken met de verwachte sterfte in de Nederlandse bevolking kwam naar voren dat volwassenen met de ziekte van Pompe een hogere sterftekans hebben. De verwachte vijfjaarsoverleving is 95%. De 10, 20 en 30-jaarsoverleving zijn respectievelijk 83, 65, en 40%. Patiënten met een ernstigere mate van handicap (Rotterdam-Handicap Scale) en die een rolstoel en/ of beademing gebruikten hebben een hoger overlijdensrisico.

Hoofdstuk 3 beschrijft de effecten en de veiligheid van het gebruik van ERT bij volwassenen met de ziekte van Pompe. In **hoofdstuk 3.1** worden de effecten van ERT zoals onderzocht in de nationale prospectieve studie beschreven, vergeleken met het ziektebeloop voor start van de behandeling. In 69 patiënten met een gemiddelde leeftijd van 52 jaar (range 26 tot 76 jaar) nam op groepsniveau de spierkracht toe met 1.4 pp/jaar zoals gemeten met gebruikmaking van de handmatige spierkrachtmeting (MRC) en met 4.0 pp/jaar voor de kwantitatieve spierkrachtmeting (HHD; hand held dynamometrie) gedurende een gemiddelde behandelduur van 23 maanden. De longfunctie in zittende houding stabiliseerde op groepsniveau, maar de longfunctie gemeten in liggende houding ging ondanks behandeling met ERT verder achteruit met -1.1 pp/jaar; (*P* =0.03). In vergelijking met het natuurlijke ziektebeloop werd gezien dat behandeling met ERT het beloop gunstig beïnvloedt. Het effect van behandeling met ERT op de spierkracht was groter bij vrouwen dan bij mannen. De longfunctie van jongere patiënten en van patiën-

ten met een betere longfunctie hadden bij aanvang van de behandeling respondeerde beter op ERT. In **hoofdstuk 3.2** beschrijven we een volwassen patiënt met de ziekte van Pompe die tijdens behandeling met ERT verder achteruit ging en infusie-geassocieerde reacties ontwikkelde. Daarnaast maakte hij ook hoge antilichaamtiters tegen het toegediende enzym aan. We toonden aan dat er zoveel antilichamen in het bloed van de betreffende patiënt aanwezig waren dat ongeveer 40% van het toegediende alglucosidase alfa gebonden werd. Bovendien toonden *in vitro* testen aan dat de opname van alglucosidase alfa negatief beïnvloed werd door de antilichaambinding. Deze resultaten wijzen op neutraliserende effecten van de antilichamen op de therapeutische werking van alglucosidase alfa. In **hoofdstuk 3.3** laten we zien dat 71 van de 73 (97%) van de volwassen patiënten antilichamen tegen alglucosidase alfa ontwikkelden. Op groepsniveau werd de hoogste antilichaamtiter (1:1259) na 6 maanden ERT gemeten. Daarna daalde de titer tot 1:270 bij een behandelduur van 36 maanden. Na een initiële stijging bleef de antilichaam titer bij het merendeel (95%) van de patiënten stabiel of daalde gedurende de verdere behandeling. Bij de patiënten met de hoogste antilichaam concentraties in het bloed trad er significante remming op van de *in vitro* alglucosidase alfa activiteit. Deze bevindingen vertaalden zich echter niet naar de klinische praktijk, omdat er geen duidelijke negatieve effecten van hoge antilichaamtiters op het behandelresultaat gezien werden. Daarentegen vonden we wel dat er bij patiënten met hogere antilichaamtiters een significant hoger risico bestond op het ontwikkelen van infusie-geassocieerde reacties. In **hoofdstuk 3.4** beschrijven we onze ervaring met ERT tijdens de zwangerschap en lactatieperiode van een 40-jarige patiënte met de ziekte van Pompe. Bij een zwangerschapsduur van 37 weken en vijf dagen werd een gezonde jongen geboren middels een keizersnede. Er waren geen maternale complicaties en zowel de intra-uteriene ontwikkeling als de ontwikkeling van het kind in de eerste levensjaren verliepen normaal. Alglucosidase alfa werd in zeer kleine hoeveelheden uitgescheiden in de moedermelk op de dag van infusie, maar 24 uur na start van de infusie was de enzym activiteit weer op het oude niveau. Op basis hiervan concludeerden we dat het veilig lijkt om behandeling met ERT te continueren tijdens de zwangerschap en de borstvoedingsperiode.

Hoofdstuk 4 gaat over vermoeidheid als veel voorkomende klacht bij patiënten met neuromusculaire ziekten. In **hoofdstuk 4.1** worden de pathofysiologische mechanismen en de beperkingen veroorzaakt door vermoeidheid bij patiënten met neuromusculaire ziekten beschreven aan de hand van de ziekte van Pompe en het Guillain-Barré syndroom. Overmatige vermoeidheid komt even vaak voor bij patiënten met neuromusculaire ziekten als bij patiënten met multiple sclerosis. Vermoeidheid kan onderverdeeld worden in vermoeidheid zoals beleefd door de patiënt en fysiologische vermoeidheid. Fysiologische vermoeidheid kan vervolgens onderverdeeld worden in centrale en perifere vermoeidheid. Bij neuromusculaire ziekten is vermoeidheid meestal van perifere origine. Echter,

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als reactie op de pathologie in het perifere zenuwstelsel kan centrale vermoeidheid ontstaan, dit om verdere schade door overbelasting van het perifere zenuwstelsel te voorkomen (central governor model). De ernst van de vermoeidheid is meestal gerelateerd aan de ernst van de onderliggende ziekte, met waarschijnlijk als enige uitzondering het monofasische Guillain-Barré syndroom. De behandeling van vermoeidheid bij neuromusculaire ziekten bestaat uit symptomatische behandeling van de onderliggende ziekte, oefentherapie en cognitieve gedragstherapie. In **hoofdstuk 4.2** beschrijven we een patiënte met een bijzondere klinische presentatie: ernstige vermoeidheid en spierpijn traden reeds op voor het ontstaan van limb-girdle spierzwakte. Opvallend was dat het spierbiopt selectieve aantasting van de type I spiervezels toonde. Mogelijk dat deze bevinding bijdraagt aan de bijzondere klinische presentatie van deze patiënte. In **hoofdstuk 4.3** wordt de mate van vermoeidheid bij 163 patiënten die deelnamen aan het IPA/Erasmus MC Pompe onderzoek gerapporteerd. De gemiddelde follow-up was vier jaar voor start van behandeling met ERT en drie jaar gedurende behandeling. Voor start van behandeling was er, over de tijd gemeten met behulp van de 'Fatigue Severity Scale' (FSS score van 5.3), geen verandering in de mate van vermoeidheid op groepsniveau. Gedurende de behandelperiode nam de FSS score met 0.13 punten per jaar af ($P < 0.001$).

In **Hoofdstuk 5** – de algemene discussie – worden de meest belangrijkste bevindingen beschreven, en worden deze vergeleken met de relevante medische literatuur en vertaald naar de implicaties voor de klinische praktijk. Daarnaast worden potentiële methoden om ERT te verbeteren, toekomstige behandelingen en suggesties voor verder onderzoek besproken.

Chapter 7

APPENDICES

APPENDIX A | STUDY DESIGN

Version adapted from the study design as described in the thesis written by N.A.M.E. van der Beek.

Study design and participants

Prospective study on the natural course and effects of enzyme replacement therapy in children and adults with Pompe disease

The Erasmus MC study on the natural course of Pompe disease and the effects of enzyme-replacement therapy (ERT) in children and adults with the disease is an ongoing, nationwide, prospective study which was initiated in 2004. All patients are seen at regular intervals at Erasmus MC University Medical Center, the designated Centre of Excellence for Pompe disease in the Netherlands. Participation is open to all children and adults diagnosed with Pompe disease. Patients with classic infantile Pompe disease are not included in this study.

Patients are recruited either through neuromuscular centres in the Netherlands and Belgium or through the Dutch neuromuscular patient organization (Spierziekten Nederland), or are referred to our Center by their treating physicians in the Netherlands or abroad. All patients or their parents/guardian gave written informed consent for participation in the study. By March 2014, 118 adults and 17 children were taking part in the study.

Since the end of 2006, alglucosidase alfa (MyozymeÒ) has officially been reimbursed in the Netherlands; from that time on, patients who had been participating in the study gradually began treatment with ERT. As the Dutch government had decided that the Center for Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center would be the only treatment center in the Netherlands, it was not possible to start treatment in all patients simultaneously. A special committee comprising neurologists, paediatricians, internal medicine specialists and geneticists was therefore instituted to decide which patients needed treatment most urgently. This decided that the most severely affected patients – meaning those who were mechanically ventilated and wheelchair-bound – should be treated first, followed by patients whose pulmonary function and muscle strength were declining rapidly. Patients in whom the disease was progressing slowly were started on ERT at a later stage. For children, the need for enzyme therapy was discussed on a case-by-case basis.

Of the Dutch patients participating in our study, 104 adults and 15 children are now being treated with enzyme therapy. Treatment is always started at our center, with all patients receiving alglucosidase alfa at a dose of 20 mg/kg every other week. If no severe side-effects are apparent within six to twelve months, patients can receive treatment

Table I Measurements performed during the natural course phase of our study in patients with Pompe disease

Procedure/measurement	t = baseline	t = every 6 months
Informed consent	X	
Inclusion/exclusion criteria	X	
General and neurological examination	X	X
Muscle strength and muscle function testing		
§ Manual muscle testing (MRC score)	X	X
§ Hand-Held Dynamometry	X	X
§ QMFT	X	X
§ AIMS[a]	X	X
§ Griffiths developmental scale[b]	X	X
Video monitoring of functional outcome measures	X	X
Pulmonary function testing		
§ Spirometry (VC, FVC, FEV_1)	X	X
§ Respiratory muscle strength (MIP, MEP)	X	X
§ Capnography	X	X
§ Ventilator use assessment[c]	X	X
Cardiac evaluation		
§ Electrocardiogram[d]	X	
§ 24-hour Holter ECG[d]	X	
§ Echocardiogram[d]	X	
Audiometry		
§ Pure-tone audiogram[d]	X	
§ Tympanography[d]	X	
§ BAEP[e]	X	
Blood sample collection		
§ CK, AST, ALT, LDH	X	X
§ Acid α-glucosidase activity in leukocytes	X	X
§ Blood film (PAS-positive lymphocyte vacuoles)	X	X
§ DNA mutation analysis	X	
Urine sample collection		
§ Oligosaccharides	X	X
Skin biopsy[f]		
§ fibroblast culture (acid α-glucosidase activity)	X	
Self-reported outcome measures		
§ Fatigue Severity Scale	X	X
§ Rotterdam Handicap Scale	X	X
§ SF-36 / TACQOL	X	X
§ R-PAct	X	X
§ HADS	X	X
§ Health-economic questionnaire	X	X

at home or at a nearby hospital. As of March 2014, 70 adults and 13 children are receiving home treatment, while 10 adults receive ERT at a hospital other than Erasmus MC University Medical Center.

As this is an ongoing study, new data were still being collected when the data from the first groups of patients had already been analysed for a specific topic. This explains the different numbers of patients described in the different chapters.

Clinical assessments and self-reported outcome measures
During the natural-course phase of the study, patients were assessed every six months. After the start of treatment, they were assessed every three months. **Tables I** and **II** give a schematic overview of the measurements performed during the study. The main investigations are described below.

Muscle strength and muscle function
The first symptoms in children and adults with Pompe disease usually involve mobility and limb-girdle weakness. A slowly progressive limb-girdle myopathy is a hallmark of the disease.

Muscle strength
Twenty-five different muscle groups were measured by manual muscle testing using the Medical Research Council (MRC) grading scale, where grade 5 represents normal muscle strength and grade 0 represents paralysis of the muscle group tested.[1] The following muscle groups were examined: neck extensors, neck flexors, abdominal muscles, trunk muscles, and bilateral sternocleidomastoid muscle, trapezius mu r adductors, shoulder abductors, shoulder exorotators, shoulder endorotators, elbow flexors, elbow extensors, wrist extensors, wrist flexors, finger extensors, finger flexors, finger abductors, hip extensors, hip flexors, hip abductors, hip adductors, knee flexors, knee extensors, ankle dorsal flexors, and ankle plantar flexors.

Table II Measurements performed during the treatment phase of our study in patients with Pompe disease

Procedure / measurement	Baseline	t = 0 weeks	t = every 2 weeks	t = 4 weeks	t = 8 weeks	t = every 3 months	t = every 6 months	t = 2 years
Informed consent	X							
Inclusion/exclusion criteria	X							
General and neurological examination	X			X	X	X		
Weight	X					X		
Infusion with alglucosidase alfa		X	X					
Vital signs	X	X	X					
Adverse events assessment		X	X					
Concomitant medication assessment		X	X					
Muscle strength/muscle function testing								
§ Manual muscle testing (MRC score)	X					X		
§ Hand-Held Dynamometry/QMT	X					X		
§ Six-minute walk test	X					X		
§ Timed tests	X					X		
§ QMFT	X					X		
§ AIMS[a]	X					X		
§ Griffiths developmental scale[b]	X					X		
Video monitoring of functional outcomes	X					X		
Pulmonary function testing								
§ Spirometry (VC, FVC, FEV_1)	X					X		
§ Respiratory strength (MIP, MEP)	X					X		
§ Ventilator use assessment[c]	X		X					
Cardiac evaluation								
§ Electrocardiogram[d]	X			X	X	X		
§ Echocardiogram[e]	X			X	X	X		
Hearing testing								
§ Tone audiogram[e]	X						X	
§ Tympanography[e]	X						X	
§ BAEP[e]	X						X	
Blood sample collection								
§ CK, AST, ALT, LDH	X			X	X	X		
§ α-glucosidase activity in leukocytes	X			X	X	X		
§ PAS-positive lymphocyte vacuoles	X			X	X	X		
§ DNA mutation analysis[f]	X							
§ Antibodies against α-glucosidase		X		X	X	X		
§ PK-analysis[g]		X						
Urine sample collection								
§ Oligosaccharides	X			X	X	X		
Skin biopsy (fibroblast culture)[f]	X							
§ acid α-glucosidase activity	X					X		

Table II *Continued*

Procedure / measurement	Baseline	t = 0 weeks	t = every 2 weeks	t = 4 weeks	t = 8 weeks	t = every 3 months	t = every 6 months	t = 2 years
X-ray spine[h]	X							
DEXA scan[i]	X							X
CT/MRI muscle[i]	X							X
Muscle biopsy[i]	X							X
Self-reported outcome measures[j]	X						X	
§ Fatigue Severity Scale	X						X	
§ Rotterdam handicap Scale	X						X	
§ SF-36/TACQOL[k]	X						X	
§ R-PAct	X						X	
§ HADS	X						X	
§ Health-economic questionnaire	X						X	

Abbreviations: *MRC= Medical Research Council; QMT= Quantitative Muscle Testing; QMFT= Quick Motor Function Test; AIMS= Alberta Infant Motor Scale; VC= Vital Capacity; FVC= Forced Vital Capacity; FEV_1= Forced Expiratory Volume in 1 second; MIP=Maximum Inspiratory Pressure; MEP= Maximum Expiratory Pressure; BAEP=Brainstem Auditory Evoked Potentials; CK= creatine kinase, AST= aspartate aminotransferase; ALT=alanine aminotransferase; LDH=Lactate dehydrogenase; PAS= Periodic Acid Schiff; DNA= Deoxyribonucleic acid; PK= pharmacokinetics; DEXA= Dual-energy X-ray absorptiometry; CT= Computed Tomography; MRI= Magnetic Resonance Imaging; SF-36= Medical Outcomes Survey Short Form-36 Health Survey; TACQOL= TNO-AZL Child Quality of Life Questionnaire; R-PAct= Rasch-built Pompe-specific Activity scale; HADS= Hospital Anxiety and Depression Scale.*
[a] In patients £ 18 months of age, [b] In patients ³ 18 months and £ 7 years, [c] If applicable, [d] If not performed prior to this study, or when abnormalities were found at a prior investigation, [e] In patients £ 5 years, [f] If not performed prior to this study, [g] Only in patients ³ 18 years. PK analysis will be performed at week 0 and week 2, [h] In children or adults with signs of scoliosis in whom corrective surgery is considered, [i] Not compulsory, [j] In patients 3 16 years unless otherwise indicated, [k] In patients ³ 6 years and £16 years.

Hand-held dynamometry (HHD) (Cytec dynamometer, Groningen, the Netherlands)[3] – which measures maximum voluntary contraction – was used to examine the following muscle groups: neck extensors, neck flexors and bilateral shoulder abductors, elbow flexors, elbow extensors, wrist extensors, hip flexors, hip abductors, knee flexors, knee extensors, ankle dorsal flexors, and ankle plantar flexors.

In a subset of patients, quantitative muscle testing (QMT) was performed.[4,5] During an isometric contraction, force was measured in the following muscle groups: bilateral shoulder adductors, elbow flexors, elbow extensors, hip adductors, knee extensors, knee flexors. Grip strength was also measured.

Muscle function

The Quick Motor Function Test (QMFT; **Appendix B**) was developed specifically to measure functional impairments in children and adults with Pompe disease.[6] Comprising 16 motor skills related to daily activities that require use of the shoulder-girdle musculature,

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trunk muscles, and pelvic-girdle/proximal lower limb muscles, this test is based on the Gross Motor Function Measure[7,8] and the IPA/Erasmus MC Pompe Survey.[9,10]

As a measure of functional endurance, we used the six-minute walk test (6MWT), which is widely regarded as an objective measure for reflecting a patient's performance in the activities of daily living (ADL).[7,8,11-13] We recorded the distance walked in six minutes. In patients who were unable to complete the full six-minute walk, we recorded the time they were able to walk.

To further evaluate the impact of muscle weakness on a patient's ability to perform functional activities in daily life, we performed three additional timed tests: 1) walking 10 meters; 2) climbing four stairs; and 3) getting up from a supine position on the floor.

Pulmonary function

As well as skeletal-muscle weakness, respiratory dysfunction is one of the main features of children and adults with Pompe disease. Severe pulmonary dysfunction may occur even in patients with minor mobility problems. We used a Lilly type pneumograph (Viasys Healthcare, Würzburg, Germany) or the KoKo spirometry system (Ferraris Respiratory, Louisville, USA) to measure vital capacity (VC), forced vital capacity (FVC), and forced expiratory volume in one second (FEV$_1$). Measurements were performed when the patient was in supine and upright seated positions.[16-18]

To obtain the maximum force that can be exerted by the respiratory muscles, we recorded maximum static inspiratory (MIP) and expiratory (MEP) pressures using a differential pressure transducer (Viasys Healthcare, Würzburg, Germany).[19]

For information on hypercapnia and alveolar hypoventilation, the carbon dioxide fraction in the expired gas was measured.[20] If patients experienced symptoms indicating nocturnal hypoventilation, they were referred to a center for home ventilation, where further investigations could determine whether mechanical ventilatory support should be started.

Safety aspects of treatment with enzyme replacement therapy

Monitoring of Infusion-Associated Reactions

Adverse or infusion-associated reactions (IARs) are any undesirable sign or symptoms experienced by a patient during the treatment period. As IARs can occur before, during and after the infusion, vital signs (blood pressure, heart frequency, breathing frequency, and temperature) were measured to guard the safety of the patient. For the first 3 months of treatment, the patients had to stay in the hospital for 1 hour after the infusion to check if any adverse reactions occur in the phase just after the infusion. After this initial

period, the patient may leave the hospital immediately after the infusion if no IARs have occurred.

In case IARs occurred the patients was physically examined, and it was judged whether the infusion needed to be temporarily interrupted or discontinued. When needed antihistamines or corticosteroids were administered to treat the symptoms associated with the IAR. With the subsequent infusion, the infusion rates were lowered for prevention of IARs. In case IARs were persisting, pre-medication (antihistamines and/or corticosteroids) was prescribed.

New symptoms and signs were documented using the adverse event form provided by Genzyme Corps.

Immunogenicity

The majority of patients with Pompe disease who are treated with ERT, developes (IgG) antibodies to alglucosidase alfa.[21,22,23] Most patients who develop antibodies do so within the first 3 months of exposure. There is evidence that classic-infantile patients developing sustained titers of anti-alglucosidase alfa antibodies have a poorer clinical response to treatment, or may lose motor function as antibody titers increase.[23,24] Therefore, we monitored antibody formation using ELISA in the majority of non-classic patients, who were receiving enzyme replacement therapy. In a subset of patients with high antibody titers, neutralizing effects of the antibodies on *in vitro* alglucosidase alfa enzymatic activity were determined.

Pregnancy and lactation

There are no adequate and well-controlled studies in pregnant women or nursing mothers. In other lysosomal storage diseases the experience with continuation of ERT during pregnancy, in much lower dosages compared to Pompe disease, did not suggest major problems.[25-29] Furthermore, the product information of alglucosidase alfa mentions normal fetal development in mice and rabbits. Based on this information, we reasoned that continuation of ERT during pregnancy was not likely to pose a substantial risk to the mother or the fetus.

During each pregnancy of a women with Pompe disease who continued to receive ERT, the clinical condition of the mother was regularly checked and fetal growth was monitored by regular ultrasound investigations. After birth the newborn baby was carefully examined by the pediatrician. Potential transfer of alglucosidase alfa in the breast milk was measured during the infusion up to 24 hours thereafter.

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Self-reported outcome measures

Rotterdam 9-items Handicap Scale (RHS)
The RHS was originally developed to measure handicap in patients with immune-mediated polyneuropathies;[30] recently it was also proven to be useful for the evaluation of patients with Pompe disease.[10] Suitable for patients age 16 years and older, the scale comprises nine questions on the following topics: mobility indoors and outdoors; kitchen tasks; domestic tasks indoors and outdoors; leisure activities indoors and outdoors; travelling; and work/study. The total score ranges from 9 to 36, with higher values representing a lower level of handicap.

Fatigue Severity Scale (FSS; Appendix C)
The FSS comprises nine items for determining the severity of fatigue and the impact of fatigue on a patient's life.[31] In recent years, it has become clear that fatigue is highly prevalent among patients with Pompe disease.[32] The total FSS score is the average of the 9 item scores and ranges from 1 ("no signs of fatigue") to 7 ("most disabling fatigue"). Scores of 4 and higher indicate fatigue, and scores of 5 and higher indicate severe fatigue. Fatigue was assessed in patients aged 16 years and older.

Quality of life
Over the last decade, greater emphasis has been placed on "quality of life" as a measure which should be included in modern studies. Quality of life in patients over 16 years was measured using the Medical Outcomes Survey short-form 36 Health Survey (SF-36; see below). The SF-36 is widely used in various health conditions, including lysosomal storage disorders.[33-35] It comprises 36 items addressing four physical health domains (physical functioning, role limitations due to physical problems, bodily pain, and general health perceptions); and four mental health domains (vitality, social functioning, role limitations due to emotional problems, and mental health). Items are summed per domain and subsequently transformed into scores between 0 and 100, with higher values representing better functioning. Its usefulness for evaluating the burden of Pompe disease was demonstrated recently.[36]

Hospital Anxiety and Depression Scale (HADS)
The HADS was developed by Zigmond and Snaith to identify anxiety disorders and depression among patients in non-psychiatric hospital clinics.[37] It is divided into an anxiety subscale (HADS-A) and a depression subscale (HADS-D), each containing seven intermingled items. All symptoms of anxiety or depression that also related to physical disorder, such as dizziness, headaches, insomnia and fatigue were excluded. The HADS

has been widely used in different disorders.[38] In our study, it was used to distinguish the fatigue caused by Pompe disease from that induced by anxiety or depression.

Rasch-built Pompe-specific Activity (R-PAct) scale

Pompe disease strongly affects patients' functioning in daily life. Although the various aspects of this are difficult to measure, it is important to quantify them, both to manage individual patients and to evaluate the possible effects of enzyme replacement therapy or any other future treatment modality. We therefore constructed a patient-based interval scale using Rasch analysis that is specifically suited to quantifying the effects of Pompe disease on a patient's ability to carry out daily-life activities.[39]

Health-economic questionnaire

The direct and indirect costs of treated and untreated Pompe disease were established through a self-applicable questionnaire, which included elements from the PROductivity and DISease Questionnaire (PRODISQ)[40], EuroQol-5D[41], and Vragenlijst Mantelzorg (Department of Public Health, Erasmus MC). We quantified seven aspects of the costs associated with medical care: living conditions, home adjustments, use of health care facilities, professional and non-professional care (e.g. partner or family), artificial ventilation, wheelchair use, and work or loss of productivity. All costs were evaluated against the background of the anticipated costs of enzyme-replacement therapy.

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APPENDIX B | QUICK MOTOR FUNCTION TEST

1. Raising the torso

Starting position: prone with arms by sides. Examiner may hold the patient's legs.

Movement: the torso must be completely raised from the mat without using the arms.

❑   initiates no neck extension
❑   initiates neck extension but cannot raise head from mat
❑   raises head from mat but torso remains on mat
❑   partially raises head and torso from the mat
❑   completely lifts head and torso from the mat (approx. 45°)

2. Neck flexion

Starting position: supine position, preferably with head in midline and arms by sides.

Movement: raises head to 45°.

❑   initiates no neck flexion
❑   initiates neck flexion (some movement of the head that indicates neck flexion such as lifting or retracting the chin) but does not raise head
❑   raises head < 45°
❑   raises head to 45° with difficulty
❑   raises head to 45° or more with no difficulty

3. Hand across midline

Starting position: supine position, preferably with head in the midline and arms by sides. Examiner holds hand at level of patient's chest on L/R sides of the midline. Asks patient to reach towards hand.

Movement: reaches with R/L arm and crosses the midline.

❑   makes no attempt to reach towards the midline
❑   makes attempt to reach towards the midline
❑   reaches with R/L arm, hand does not cross the midline
❑   reaches with R/L arm, hand crosses the midline with difficulty (slowly, requiring effort)
❑   reaches with R/L arm, hand crosses the midline without difficulty

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4. Hip and knee flexion

Starting position: supine position, preferably with head in the midline, legs extended and arms by sides.

Movement: flexes R/L hip and knee through full range of motion

- ❏   unable to initiate flexion in R/L hip and knee
- ❏   initiates flexion in R/L hip and knee, but does not move hip more than 10°
- ❏   flexes R/L hip and knee through part of full range of motion(<90°)
- ❏   flexes R/L hip and knee through full range of motion but with difficulty (slowly, with effort)
- ❏   without difficulty flexes R/L hip and knee through full range of motion

5. Extending the legs

Starting position: supine position, preferably with head in the midline, legs stretched and arms by sides.

Movement: extends and raises both legs simultaneously.

- ❏   does not attempt to raise legs
- ❏   attempts to raise legs but neither leg leaves the mat (tightens abdominal/leg muscles), or raises 1 leg
- ❏   raises legs from mat but does not extend them, or uses arms
- ❏   with difficulty extends both legs and lifts them from the mat (e.g. very briefly)
- ❏   extends both legs and lifts them from the mat without difficulty

6. Sit up

Starting position: supine position, preferably with head in the midline, legs in comfortable position, arms by sides or crossed over the chest.

Movement: sit up without support

- ❏   does not attempt to sit up (or initiate neck flexion)
- ❏   attempts, but does not achieve sit up (even using the arms)
- ❏   does a sit up, but using the arms
- ❏   does a sit up without using arms but with difficulty
- ❏   does a sit up without using arms without difficulty (quick and controlled movement)

7.   Extending the arms

Starting position: comfortable sitting position (seated on a chair, not leaning on back of the chair), arms by sides.

Movement: raises both arms upwards along the body (180°).

❑   does not attempt to raise arms

❑   attempts to raise arms but they do not come above shoulder level

❑   raises both arms above shoulder level but arms do not quite reach 180 °

❑   raises both arms along the body and hands touch above the head but with difficulty (arms are not completely stretched)

❑   raises both arms along the body and hands touch above the head without difficulty (arms remain extended)

8.   Standing up from a chair

Starting position: seated in a chair, arms by sides not leaning on the back of the chair.

Movement: stands up from chair without using arms.

❑   makes no attempt to stand up from chair

❑   attempts to stand up from chair but is not able to (even using arms)

❑   stands up from chair using arms

❑   stands up from chair without using arms but with difficulty (slowly, with effort, number of attempts are necessary)

❑   stands up from chair without using arms without difficulty

9.   Standing up from half-knee

Starting position: kneeling without arm support.

Movement: stands by means of half-knee position using L/R knee without using arms.

❑   makes no attempt to stand, OR: non-applicable

❑   attempts to stand up but is not able to (even using arms)

❑   stands by means of half-knee position using L/R knee and using arms

❑   stands by means of half-knee position using L/R knee without using arms but with difficulty

❑   stands by means of half-knee position using L/R knee without using arms and without any difficulty

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10. Squatting

Starting position: standing.

Movement: squats without using arms.

❑   does not initiate squat, or: non-applicable

❑   initiates squat but is unable to bend legs to 90° (even using arms or a support)

❑   able to squat using arms or holding on

❑   able to squat without using arms, but with difficulty (quickly falls over, cannot easily maintain position)

❑   squats with no difficulty without using arms

11. Standing up from a squatting position

Starting position: squatting:

Movement: stands without using arms.

❑   unable to stay in squatting position without help, OR: non-applicable

❑   attempts to get up from squatting position but is unable to stand (also not when using arms)

❑   able to go from squatting to standing using arms

❑   able to go from squatting to standing without using arms but with difficulty

❑   goes from squatting to standing without using arms with no difficulty

12. Picking up an object

Starting position: standing without arm support.

Movement: able to pick up an object from the floor and stand up again without arm support.

❑   makes no attempt to pick up an object from the floor, OR: non-applicable

❑   attempts to pick up an object from the floor, put does not pick up the object

❑   picks up object from the floor and stands up again using arms (uses arms for balance, both on the floor and on the body)

❑   able to pick up an object from the floor without arm support and stand up again with difficulty

❑   able to pick up an object from the floor without arm support and stand up again without difficulty (fast, controlled movement)

13.   Standing on one leg
Starting position: standing without arm support
Movement: standing without arm support, lift L/R foot up for 10 seconds (and remains standing on the same leg).
❑   lifts L/R foot up without arm support, OR: non-applicable
❑   stands without arm support, lift L/R foot up for < 3 seconds
❑   stands without arm support, lifts L/R foot up for 3-9 seconds
❑   stands without arm support, lifts L/R foot up for 10 seconds with difficulty
❑   stands without arm support, lifts L/R foot up for 10 seconds without difficulty

14.   Walking ten metres
Starting position: standing without arm support.
Movement: walks forward for 10 metres without arm support
❑   does not attempt to walk, OR: non-applicable
❑   attempts to, but cannot walk for 10 m, even with support (hands, wall)
❑   walks 10 m but uses hands or wall for support
❑   walks 10 m without support of hands or wall, but with abnormal gait (e.g. staggering)
❑   walks 10 m without difficulty

15.   Jumping
Starting position: standing without arm support.
Movement: jumps forward with both feet simultaneously
❑   does not attempt to jump forwards, OR: non-applicable
❑   jumps forwards < 10 cm with both feet simultaneously (or falls on jumping or landing)
❑   jumps forwards between 10 and 40 cm with both feet simultaneously
❑   jumps forwards between 40 and 100 cm with both feet simultaneously
❑   jumps forwards more than 100 cm with both feet simultaneously and without effort

16.   Walking up steps
Starting position: standing without arm support.
Movement: walks up 4 steps using alternating feet without arm support
❑   does not attempt to walk up 4 steps, OR: non-applicable
❑   walks (alternating or non-alternating feet), up 1 or more step using railing
❑   walks (alternating or non-alternating feet), up 4 steps using railing
❑   walks up 4 steps using non-alternating feet without arm support
❑   walks up 4 steps using alternating feet without arm support

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APPENDIX C | FATIGUE SEVERITY SCALE

Individuals are instructed to choose a number from 1 to 7 that indicates their degree of agreement with each statement: 1 indicated strongly disagree and 7 strongly agree.

	Completely Disagree					Completely Agree	
1. My motivation is lower when I am fatigued	1	2	3	4	5	6	7
2. Exercise brings on my fatigued	1	2	3	4	5	6	7
3. I am easily fatigued	1	2	3	4	5	6	7
4. Fatigue interferes with my physical functioning	1	2	3	4	5	6	7
5. Fatigue causes frequent problems for me	1	2	3	4	5	6	7
6. My fatigue prevents sustained physical functioning	1	2	3	4	5	6	7
7. Fatigue interferes with carrying out certain duties and responsibilities	1	2	3	4	5	6	7
8. Fatigue is among my 3 disabling symptoms	1	2	3	4	5	6	7
9. Fatigue interferes with my work, family or social life	1	2	3	4	5	6	7

Krupp et al. 1989

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EPILOGUE

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Abbreviations

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Verwijzende artsen
J.H.J. Wokke, M. de Visser, B.G.M. van Engelen, J.B.M. Kuks, A.J. van der Kooi, N.C. Notermans, C.G. Faber, J.J.G.M. Verschuuren, W.L. van der Pol, G.J. Biessels, H.J. Schelhaas, A.R. Wintzen, W.J.H.M. Grosveld, G. Hageman, A.M. Boon, H. Kerkhof, L.G.F. Sinnige, N.M. Vlam, J.T.H. van Asseldonk, A. van der Zwart, E.S.C. Korf, J.C. Doelman, A.J. Breukelman, M.F.G. van der Meulen, J. Trip, M.E. Pouwels, G. de Jong, C. Bouwsma, P.R. Schiphof, N. Voermans, J. de Bleecker, P.W. Wirtz, H.G.J. Dieks, en M.H.F. Bongers.

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Collega's van het Centrum voor Lysosomale en Metabole Ziekten

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ABOUT THE AUTHOR

Juna de Vries was born on August 8th, 1980 in Wageningen, The Netherlands. After passing her finals at the Wagenings Lyceum in 1999, she started her medical training at the Faculty of Medicine of the University Medical Center Utrecht. During her studies, she participated in the end-of-life decision making research project in Amyotrophic Lateral Sclerosis under the supervision of Prof. dr. L.H. van den Berg at the Department of Neurology of the same Center. After obtaining her medical degree in 2006, she temporarily worked as a research physician at the Utrecht Institute for Pharmaceutical Sciences, Division of Pharmacology of Utrecht University (head: Prof. dr. B. Olivier). From 2006 until 2008 she worked as a medical doctor at the Department of Neurology at both the Erasmus MC University Center (head: Prof. dr. P.A.E. Sillevis Smitt) in Rotterdam and the University Medical Center Utrecht (head: Prof. dr. J.H.J. Wokke). In 2008 she was offered the opportunity to succeed Nadine van der Beek at the research project underlying this thesis concerning Pompe disease under the supervision of Prof. dr. P.A. van Doorn, Prof. dr. A.T. van der Ploeg, and dr. A.J.J. Reuser at the Center for Lysosomal and Metabolic Diseases, Erasmus MC University Medical Center. From 2011 onward she works as a resident in Neurology at the Erasmus MC University Medical Center (head: Prof. dr. P.A.E. Sillevis Smitt).

Currently, she and her partner Eric van Breda live in Rotterdam and last October they became the proud parents of their lovely son Fedja.

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PUBLICATIONS

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9. **de Vries JM**, van der Beek NA, Hop WC, Karstens FP, Wokke JH, de Visser M, van Engelen BG, Kuks JB, van der Kooi AJ, Notermans NC, Faber CG, Verschuuren JJ, Kruijshaar ME, Reuser AJ, van Doorn PA, van der Ploeg AT. Effect of enzyme therapy and prognostic factors in 69 adults with Pompe disease: an open-label single-center study. *Orphanet J Rare Dis* 2012;26(7):73.

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PHD PORTFOLIO

Summary of PhD training	Year	Workload (ECTS)
General courses		
Principles of Research in Medicine and Epidemiology	2008	0.7
Introduction to Data-analysis	2008	0.9
Classical methods for data-analysis	2008	5.7
Modern statistical methods	2009	4.3
Regression analysis	2009	1.9
Repeated measurements	2010	1.4
Biomedical English writing and communication	2010	3.0
International conferences		
Symposium on Lysosomal Storage Disorders, Paris	2008	0.5
World Muscle Society, Geneva (oral presentation, 2 poster presentations)	2009	2.0
World Muscle Society, Almancil (3 poster presentations)	2011	1.5
Steps Forward in Pompe disease, Munich (oral presentation, 2 poster presentations)	2009	2.0
Steps Forward in Pompe disease, London (Poster presentation)	2010	0.5
Steps Forward in Pompe disease, Budapest (Oral presentation, 3 poster presentations)	2011	2.5
International Symposium of the Portuguese Society of Metabolic Disorders (SPDM), Algarve (oral presentation)	2010	1.0
Seminars and workshops		
Belgisch-Nederlandse neuromusculaire studieclub, Leuven	2008	0.2
Belgisch-Nederlandse neuromusculaire studieclub, Utrecht	2009	0.2
Belgisch-Nederlandse neuromusculaire studieclub, Utrecht (oral presentation)	2010	1.0
Belgisch-Nederlandse neuromusculaire studieclub, Utrecht	2011	0.2
Belgisch-Nederlandse neuromusculaire studieclub, Utrecht	2012	0.2
European NeuroMuscular Centre (ENMC) workshop, Naarden (oral presentation)	2010	1.0
Reunião da Primavera da Sociedade Portuguesa de Estudos de Doenças, Coimbra (oral presentation)	2011	1.0
National symposium on Pompe disease, Thessaloniki	2011	1.0
In depth courses		
International Postgraduate Course on Lysosomal Storage Diseases, Nierstein	2008	1.0
Pompe disease expert day, Rotterdam (3 oral presentations)	2007-2010	3.0
Research conference on Pompe disease, Boston (2 oral presentations)	2010	2.0
Boerhaave Prinses Beatrix Fonds symposium neuromusculaire ziekten	2009-2013	0.8

Other

Amicus investigators' meeting POM-CL-201, Enniskerry	2008	0.5
Presentation, media and communication coaching by LionsDen, London	2010	0.5

Teaching activities

Teaching of 2nd year medical students at the Utrecht University Center	2008-2010	3.0
Training homecare nurses, Rotterdam	2011	1.0
Reviewing papers for peer reviewed journals	2010-2013	1.0

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ABBREVIATIONS

4-MU	4-methylumbelliferyl-α-D-glucopyranoside
6MWT	Six-minute walk test
α-Glu	Acid α-glucosidase
ACh	Acetylcholine
AIMS	Alberta infant motor scale
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ANA	Antinuclear antibodies
ATPase	Adenosine triphosphatase
BAEP	Brainstem auditory evoked potentials
BSA	Bovine serum albumin
CAF	Central activation failure
CBS	Dutch central bureau of statistics
CCMO	Central committee on research involving human subjects in the Netherlands
CCV	Clathrin-coated vesicles
CHO-cells	Chinese hamster ovary-cells
CI	Confidence interval
CIDP	Chronic inflammatory demyelinating polyneuropathy
CIS	Checklist individual strength
CK	Creatine kinase
CNS	Central nervous system
CMT	Charcot-Marie-Tooth disease
COX	Cytochrome oxidase
CRIM	Cross reactive immunogenic material
CT	Computed tomography
CTG	College tarieven gezondheidszorg
CVZ	College voor zorgverzekeringen
DEXA	Dual-energy X-ray absorptiometry
DNA	Deoxyribonucleic acid
EB	Empirical Bayes estimates
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
EMA	European medicines agency
eow	Every other week
ERT	Enzyme replacement therapy

FcR	Fragment crystallisable receptor
FDA	Food and drug administration
FEV_1	Forced expiratory volume in 1 second
FIS	Fatigue Impact Scale
FSHD	Facioscapulohumeral Muscular Dystrophy
FSS	Fatigue severity scale
FVC	Forced vital capacity
GAA	Acid α-glucosidase or gene coding for acid α-glucosidase
GBS	Guillain–Barré syndrome
GSD	Glycogen storage disease
HADS	Hospital anxiety and depression scale
HE	Hematoxylin and eosin staining
HHD	Hand-held dynamometry
HP-GAA	Hyper-mannose-6-phosphorylated variant of recombinant human acid α-glucosidase generated by enzymatic engineering
HSCT	Hematopoietic stem-cell transplantation
IAR	Infusion-associated reaction
ICF	International classification of functioning, disability and health
IgE	Immunoglobulin E
IGF-2	Insulin growth factor 2
IgG	Immunoglobulin G
IPA	International Pompe Association
IQR	Inter quartile range
ISNO	Interuniversitair steunpunt neuromusculair onderzoek (the Dutch neuromuscular research center)
IVS1	Common splice-site mutation in non-classic Pompe disease IVS1-13T>G (c.-32-13T>G)
LDH	Lactate dehydrogenase
kDa	Kilo Dalton
LEMS	Lambert-Eaton myasthenic syndrome
LGMD	Limb-girdle muscular dystrophy
LOTS	Late-onset treatment study
M6P	Mannose-6-phosphate
MC	Medical center
MEP	Maximal expiratory pressure
MFI	Median fluorescence intensity or multidimensional fatigue inventory
MFM	Motor function measure
MIP	Maximal inspiratory pressure

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MMT	Manual muscle testing
MPS	Mucopolysaccharidosis
MRC	Medical Research Council
MRI	Magnetic resonance imaging
N or n	Number of patients
NA	Not applicable
NADH	Nicotinamide adenine dinucleotide
neo-rhGAA	Hyper-mannose-6-phosphorylated variant of recombinant human acid α-glucosidase generated by chemical conjugation of synthetic oligosaccharides bearing M6P residues
NR	Not reported
OMIM	Online Mendelian Inheritance in Man
OPMD	Oculopharyngeal muscular dystrophy
ORO	Oil red O
DM	Myotonic dystrophy
PAS	Periodic acid-Schiff
PBS	Phosphate buffered saline
PCS	Physical Component Summary
PEDI	Pediatric evaluation of disability inventory
PK	Pharmacokinetics
PNS	Peripheral nervous system
pp	Percentage points
PROMM	Proximal myotonic myopathy
QoL	Quality of life
QMFT	Quick motor function test
QMT	Quantitative muscle testing
rAAV	Recombinant adeno-associated viruses
R-FSS	Rasch-build fatigue severity scale
rhGAA	Recombinant human acid α-glucosidase
RHS	Rotterdam 9-items handicap scale
RIP	Radio-immuno precipitation assay
R-PAct	Rasch-built Pompe-specific Activity scale
SD	Standard deviation
SDH	Succinate dehydrogenase
SEPN1RM	Selenoprotein N1-related myopathy
SF-36	Medical outcomes study short-form 36 health survey
sIBM	Sporadic inclusion body myositis
SMA	Spinal muscular atrophy

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TACQOL	TNO-AZL child quality of life questionnaire
TMB	3,3',5,5'–tetramethylbenzidine
TSH	Thyroid-stimulating hormone
VAS-F	Visual analogue scale for fatigue
VC	Vital capacity
VPRIV	Velaglucerase alfa for injection
VSN	Vereniging Spierziekten Nederland
WGM	Walter Gardner scale
WHO	World health organization

Chapter

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