The Musculoskeletal System in Pompe disease

Pathology, consequences and treatment options

Linda van den Berg

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The Musculoskeletal System in Pompe Disease

Pathology, Consequences and Treatment Options

Het musculoskeletaal stelsel bij de ziekte van Pompe

Pathologie, consequenties en behandelmogelijkheden

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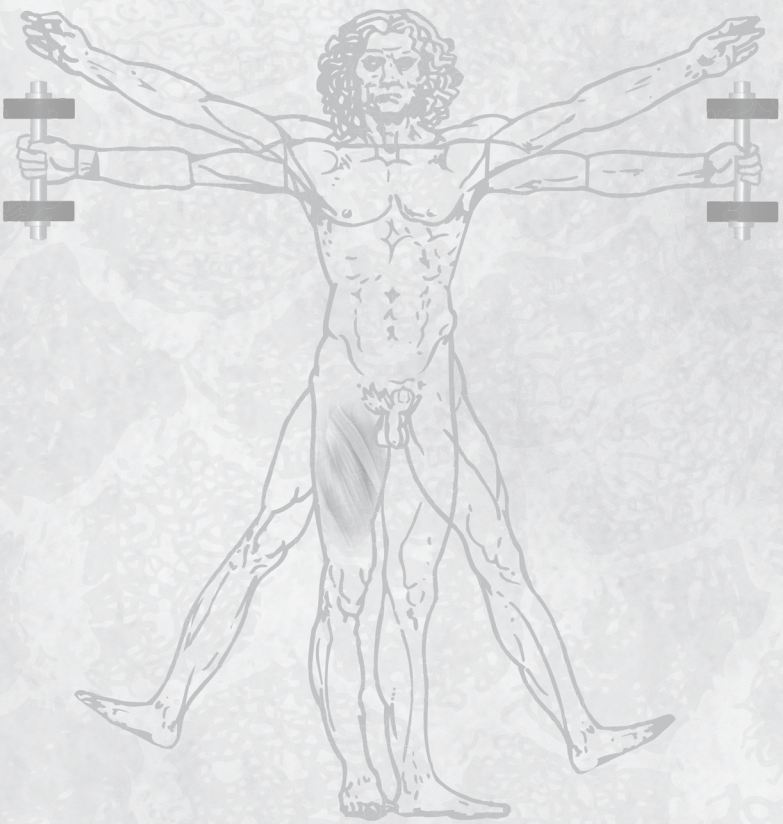
CHAPTER 1

General introduction & Scope of the thesis



Pompe disease, also known as glycogen storage disorder type II and acid maltase deficiency, is a rare metabolic myopathy. It is caused by a deficiency of lysosomal acid α-glucosidase which results in the accumulation of glycogen in cells, especially muscle cells. The disease presents as a broad clinical spectrum with progressive muscle weakness as prominent symptom and can hence be categorized as a lysosomal storage disorder, a glycogen storage disorder and also as a neuromuscular disorder.

The broad term "neuromuscular disorders" encompasses many different syndromes and diseases that either directly or indirectly impair the function of the skeletal muscles, the muscles that enables to move the limbs and trunk. Myopathies, diseases of the muscle itself, are a subgroup of neuromuscular disorders and include muscular dystrophies, inflammatory disorders of the muscle and metabolic diseases of the muscle.

Pompe disease was the first myopathy for which treatment became available. Enzyme replacement therapy (ERT) is the registered treatment for Pompe disease. It was shown to elicit positive effects first in babies with the most severe form of the disease[1-5] and later also in children and adults with more slowly progressive forms of the disease.[6-16] Despite improvements in skeletal muscle strength, walking distance, respiratory function and survival, not all patients respond equally well to treatment and not all muscle damage and functional impairment is resolved.[14,17,18] Long-term clinical programs should therefore also focus on co-morbidities and additional strategies to improve patients' functioning.

This thesis explores the muscle pathology in Pompe disease across the clinical spectrum, the interplay between skeletal muscle dysfunction and bone structure and the effect of enzyme therapy on it, as well as the use of exercise training to improve patients' functioning.

This introductory chapter gives an overview of the history, pathophysiology, clinical characteristics, diagnosis and treatment of Pompe disease and reviews the use of exercise training programs in myopathies.

HISTORY OF POMPE DISEASE

In 1932 the Dutch pathologist Johannes Cassianus Pompe reported his findings in a 7-month-old girl who had succumbed to hypertrophic cardiomyopathy. His microscopic findings showed large amounts of vacuolar glycogen accumulation not only in the heart, but also in the liver, kidneys and skeletal muscles. The disease was named after him and was later classified as glycogen storage disease type II.[19]

In the fifties the Belgian cell biologist Christian de Duve described the lysosome, a subcellar membrane-bound organelle that contains an array of enzymes capable of breaking down waste materials and cellular debris,[20] for which discovery he received the Nobel Prize in Physiology or Medicine in 1974. This allowed acid α-glucosidase to be identified by Henri-Géry Hers in 1963 as the first of many lysosomal enzymes.[21] Hers linked the deficiency of acid α-glucosidase to five patients with infantile onset Pompe disease. Pompe disease thereby became the first lysosomal storage disorder with a known cause.

During the 1960s and 1970s, acid α-glucosidase deficiency accompanied by lysosomal glycogen storage was described in patients much older than those reported by Dr. Pompe and Hers. Andrew Engel introduced the name acid maltase deficiency to make a distinction between patients with onset of symptoms in late childhood or adulthood and infants with symptoms manifesting shortly after birth.[22-24]

Currently, the name Pompe disease encompasses the entire clinical spectrum ranging from onset at birth till late adulthood. In my thesis I will adhere to the terminology proposed by Gungor and Reuser, dividing the Pompe patient population into classic infantile Pompe disease, i.e. onset of symptoms within the first year of life in combination with hypertrophic cardiomyopathy and virtually total lack of acid α-glucosidase activity, and non-classic Pompe disease for all other patients.[25]

Disease pathogenesis and pathophysiology

Pompe disease is characterized by a total or partial deficiency of the lysosomal enzyme acid α-glucosidase with its gene (*GAA*) located on chromosome 17. Currently, more than 300 pathogenic mutations have been identified (www.pompecenter.nl). In the majority of Caucasian children and adults with Pompe disease a single splice-site mutation, c.-32-13T>G (IVS1) is responsible for the disease.[26-28]

Fully functional acid α-glucosidase degrades lysosomal glycogen into glucose (Figure 1A). In patients with Pompe disease, however, the deficiency of acid α-glucosidase causes glycogen to accumulate in the lysosomes (Figure 1B).[29,30]

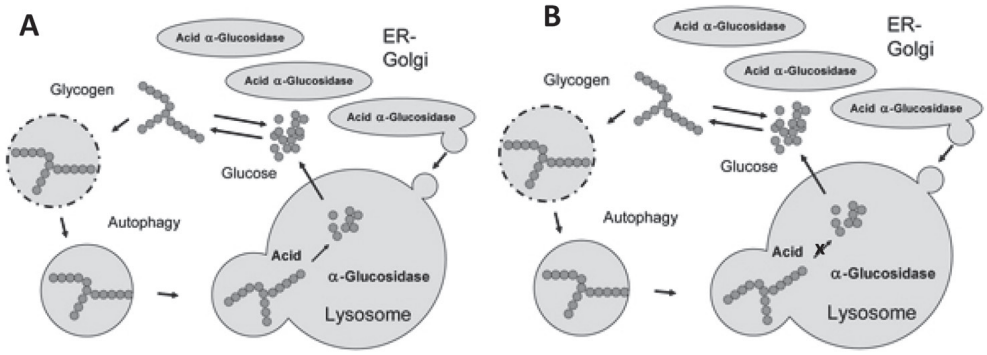
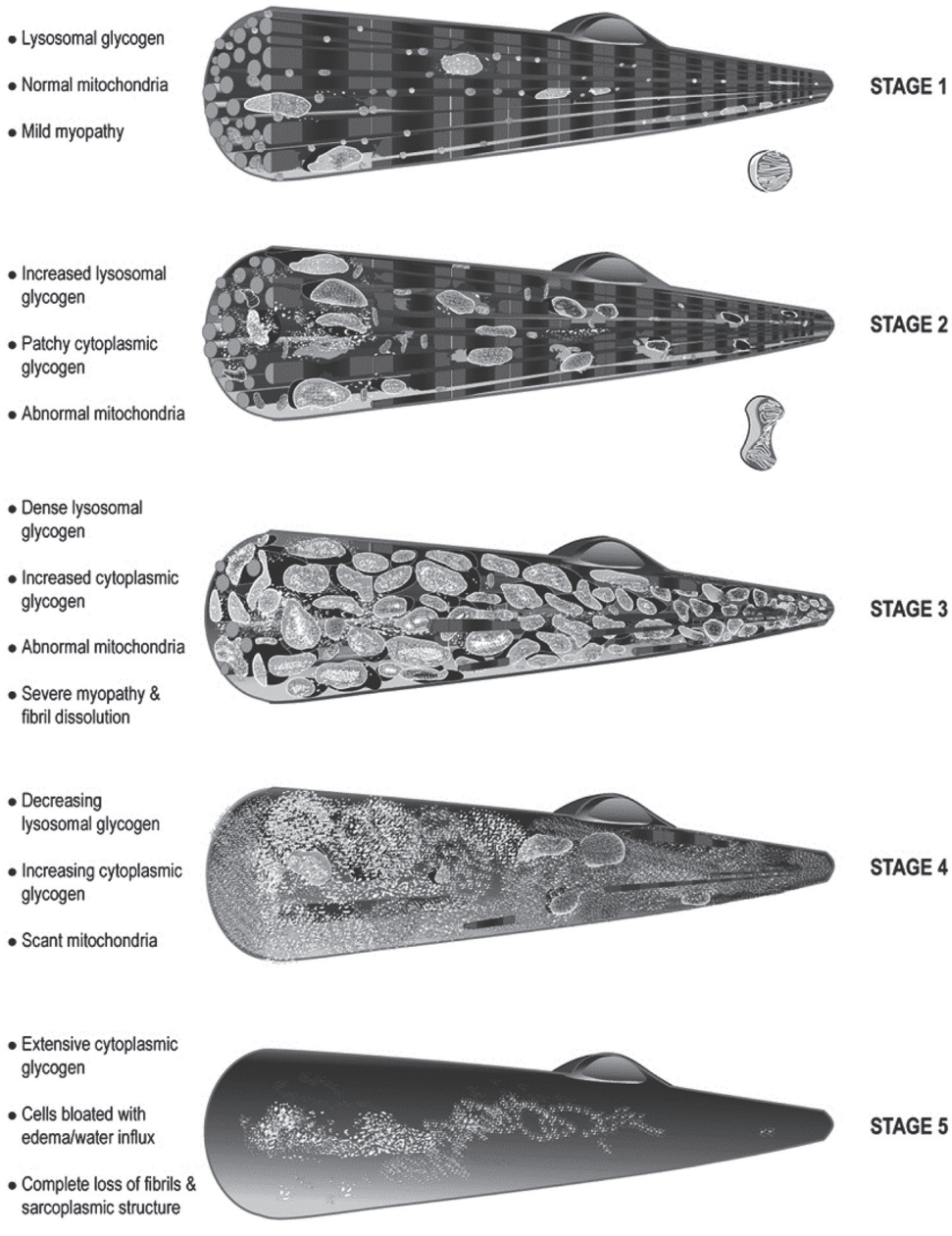


Figure 1 | Fully functional acid α-glucosidase degrades lysosomal glycogen into glucose (A).In patients with Pompe disease the deficiency of acid α-glucosidase causes glycogen to accumulate in lysosomes (B).

This leads to an increase in their size and number (Figure 2).[31] which by itself causes mechanical loss of muscle contractility and muscle weakness.[32] The simultaneous overexpression of desmin and titin, two proteins that normally enforce the muscle cell structure, is possibly a mechanism to compensate for the loss of muscle strength.[33,34] In addition, the lysosomal membranes can rupture due to the ongoing glycogen accumulation, whereby the lysosomal content is released into the cytoplasm and causes direct damage to the contractile elements (Figure 2).[31] Autophagic build up also contributes to the pathogenic process: large fields of centrally localized autophagic debris can be found in the muscle fibers of patients with Pompe disease.[22,29,35-38] Autophagy refers to a process whereby components of the cell itself are wrapped in membranes and transported to the lysosomes for re-cycling purposes. In Pompe disease, this process is stalled by dysfunction of the overloaded lysosomes, and autophagic build-up occurs.[33,39,40] It is mainly seen in type 2 muscle fibers.[39,41]

Energy shortage was once considered as contributing factor to the muscle weakness, and the increased amounts of lipofuscin seen in muscle biopsies indicates that oxidative stress occurs.[33,42] On the other hand, studies on energy expenditure in Pompe disease have shown that the skeletal muscle glycogenolytic capacity is sufficient, even during exercise.[43-45] Thus, exercise intolerance and oxidative stress are more likely consequences of the skeletal muscle weakness, due to structural changes, than the result of metabolic changes.[43]

Figure 2 | Cartoon illustration of ultrastructural disease progression in five stages.



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Disease presentation and clinical spectrum

Patients with classic infantile Pompe disease, the phenotype originally described by Dr. Pompe in 1932, usually present with severe hypotonia, feeding difficulties and failure to thrive within the first months of life.[1,46] Their motor development is delayed and major developmental milestones are usually not reached.[47] Their heart is characteristically enlarged and death typically occurs in the first year of life due to cardiorespiratory failure.[46,48] Further symptoms include hearing loss, speech difficulties and facial muscle weakness.[49-51]

In other forms of Pompe disease, the heart is not prominently involved and the muscle weakness progresses slower. These patients suffer most from limited mobility and respiratory insufficiency, which can ultimately lead to wheelchair use and artificial ventilation.[24,47,52-54] The skeletal muscle weakness typically fits a pattern of limb-girdle myopathy with the abdominal muscles, paraspinal muscles, hip flexors, hip extensors, hip adductors and hip abductors most severely affected.[55] Weakness of those muscles leads to difficulty with walking, climbing stairs and rising from the floor.[54-56] Children and adults with Pompe disease also complain of fatigue[57] and pain.[57,58] Some have ptosis, bulbar muscle weakness and/or scapular winging.[55,59-61]

To a certain extent, the clinical presentation depends on the level of residual acid α-glucosidase activity, which is determined by the type of mutations in each of the two copies of the *GAA* gene. In classic infantile Pompe disease acid α-glucosidase activity is absent, while in patients with non-classic phenotypes some residual enzyme activity is always detectable. However, among patients with non-classic phenotypes the genotype-phenotype correlation is weak; even patients with the same *GAA* haplotypes may have a quite different age of disease onset. Clinical variation also occurs within families with the same genotype.[62] It shows that epigenetic and environmental factors co-determine the Pompe disease phenotype next to the *GAA* genotype.[47,63]

Diagnosis

Diagnosing Pompe disease is often difficult, partly because it is a rare disorder and partly because its clinical presentation can resemble that of other diseases. Routine clinical examination and testing may help to differentiate Pompe from other diseases. The biochemical determination of the level of acid a(alpha)-glucosidase activity and/or mutation analysis of the gene for acid a-glucosidase are needed to provide a definitive diagnosis.

On physical examination, patients with classic infantile Pompe disease show 'slipping through' on vertical suspension and have a prominent head lag. Tendon reflexes are often decreased. Additional features can be enlargement of the tongue and moderate enlargement of the liver.[47] A chest X-ray and electrocardiography (ECG) reveals cardiac involvement. In 80%

of all cases of non-classic Pompe disease physical examination will reveal weakness of the shoulder abductors, abdominal muscles, paraspinal muscles and/or hip muscles.

Routine laboratory testing in all cases of Pompe disease may show elevation of serum levels of creatine kinase (CK), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH), although (a small minority of) patients can also have levels within the normal range.[18,48,55,64,65] Pulmonary function tests may reveal decreased forced vital capacity (FVC).[66] and needle electromyography (EMG) may show myopathic patterns.[67]

The best way to confirm the diagnosis Pompe disease is to measure the acid alpha-glucoisdase activity in leukocytes or fibroblasts of the patient.[30,68] A first indicative test can be performed on a bloodspot sample[69-71] or the newer dried blood spot test can be used.[72-75] Although muscle biopsy is an invasive procedure and diagnosis on biopsies is not advised because of the low acid α-glucosidase activity in skeletal muscle, it does provide the opportunity to assess pathological changes. In classic infantile Pompe disease, the muscle pathology closely parallels the clinical findings. In patients with other phenotypes, the diagnostic value of muscle biopsies is rather limited, and some biopsies may not even show signs of glycogen storage.[17,42,56,76]

Enzyme replacement therapy

Pompe disease is a lysosomal storage disorder, and macro-molecular compounds offered to cells can find their way to the lysosomes through a process called endocytosis. Hereby, a product binds to the surface of the cell whereupon the cell membrane invaginates locally so that an intra-cellular vesicle -an endosome- emerges containing the compound. Subsequently, the endosome fuses with the lysosomes and delivers its content to the lysosomes.[77] Soon after the discovery of the lysosomal enzyme defect in Pompe disease it was speculated that the naturally occurring endocytic pathway could be employed to deliver the missing enzymes to patients with lysosomal storage diseases.[78] Development of enzyme therapy for Pompe disease went into high gear after cloning the *GAA* gene, and the design of production systems for recombinant human acid alpha-glucosidase.[79-83] In 1999 the first clinical trial in infants was performed at the Erasmus MC University Medical Center.[1,84,85] The six infants who participated in this trial were treated with recombinant human acid α-glucosidase (rhGAA) extracted from the milk of transgenic rabbits. Only few months later, another trial was started in the USA in three infants whereby genetically engineered Chinese Hamster Ovary (CHO) cells were used as source of rhGAA. The results of these first trials followed by others in patients with classic infantile Pompe disease led to approval of the application of alglucosidase alfa (rhGAA / Myozyme®) by both the European and United States regulatory authorities (EMA and FDA) in 2006.

Table 1 | Summary of clinical trials of exercise training in children and adults with myopathies

Author	Year of publication	Kind of myopathy	No. of patients	Exercise training
Muscular Dystrophies				
Vignos et al.	1996	Duchenne	14	Resistance
		LGMD	6	Resistance
Lindeman et al.	1995	MD	33	Resistance
Tollback et al.	1999	MD	6	Resistance
Olsen et al.	2005	FSHD	8	Aerobic
Orngreen et al.	2005	MD	12	Aerobic
Sveen et al.	2007	LGMD	9	Aerobic
Sveen et al.	2008	Becker	11	Aerobic
Mitochondrial and Metabolic Myopathies				
Taivassalo et al.	1998	Mitochondrial	10	Aerobic
Cejudo et al.	2006	Mitochondrial	20	Aerobic + Resistance
Jeppesen et al.	2006	Mitochondrial	20	Aerobic
Taivassalo et al.	2006	Mitochondrial	8	Aerobic
Terzis et al.	2011	Metabolic	5	Aerobic + Resistance
Inflammatory Myopathies				
Escalante et al.	1993	PM + DM	5	Resistance
Wiesinger et al.	1998	PM + DM	14	Aerobic + Resistance
Alexanderson et al.	1999	PM + DM	10	Aerobic + Resistance
Arnadottir et al.	2003	IBM	7	Aerobic + Resistance
Dastmalchi et al.	2007	PM + DM	9	Aerobic + Resistance

Table 1 | *Continued*

Effect on:				
Endurance	**Muscle strength**	**Muscle function**	**Fatigue**	**Muscle biopsy**
Muscular Dystrophies				
–	Improvement	Timed tests: decliine Walking: stable	–	–
–	Improvement	Timed tests: stable Walking: stable	–	–
–	Stable	Timed tests: stable Self-reported: stable	Stable	–
–	Improvement	–	–	Damage: stable Distribution: type 1↑
Improvement	–	–	Stable	Distribution: unchanged Size: unchanged
Improvement	–	–	Stable	Distribution: unchanged Size: type 1 + 2a ↑
Improvement	–	–	Stable	Distribution: unchanged Size: unchanged
Improvement	Improvement	–	–	Damage: stable
Mitochondrial and Metabolic Myopathies				
Improvement	–	SF-36: improvement	–	–
Improvement	Improvement	Walking: stable	–	–
Improvement	–	Walking: stable	stable	Distribution: unchanged Damage: stable mtDNA: unchanged
Improvement	–	–	–	Damage: stable mtDNA: unnchanged
Improvement	Improvement	Stable	–	–
Inflammatory Myopathies				
–	Improvement	–	–	–
Improvement	Improvement	Self-reported: improvement	–	–
–	Improvement	Improvement SF-36: improvement	–	Inflammation: stable
–	Stable	–	–	Inflammation: stable
–	–	improvement	–	Distribution: type 1↑ Size: type 2 ↑

Since then clinical studies in children and adults have been performed confirming the beneficial effects of ERT in all forms of Pompe disease, making Pompe disease the first treatable myopathy. A recent review about the effects of enzyme therapy, containing data from 368 patients reports that the large majority of patients (about two-thirds) stabilize or improve with regard to respiratory and motor function. The most obvious effect of ERT in patients with classic infantile Pompe disease is that the therapy prolongs their lives significantly. It was recently shown that also adults have a longer life expectancy when receiving enzyme therapy.[15]

Despite the positive experiences with the application of ERT in Pompe disease, improvement of the therapeutic effect remains desired.[6-16] Not all muscle damage and functional impairment is resolved,[14,17,18] and not all children and adults benefit equally well from therapy. A recent study in 69 adult patients suggested that female gender, younger age and better clinical status are favourable prognostic factors for the effect of ERT. Further research is required to identify the epigenetic and environmental factors that co-determine the clinical course of Pompe disease. Long-term clinical and therapeutic follow-up should focus on co-morbidities and strategies to improve patients' remaining functional impairments.

Exercise training in Pompe disease and other myopathies

Patients' fitness and physical functioning may also be improved by more general approaches such as exercise training. An exercise training program can contain different components of physical fitness to achieve specific goals, e.g.:

1. Aerobic training, which leads to improvements in endurance by one or a combination of improvements in respiration, cardiac function, central circulation, peripheral circulation, and skeletal muscle metabolism. Exercises include walking, swimming, cycling, rowing etc.
2. Strength training improves the maximal force development capacity of a skeletal muscle by increases in muscle size and muscle strength due to the formation of new contractile elements. Strength training can be focussed on mainly muscle strength, e.g. to contract a muscle maximal for a short period of time or can also be focussed on strength endurance, e.g. to contract a muscle with a specific force for a sustained period of time. Exercises are performed with weights and are mainly focussed on specific muscles or muscle groups.
3. Core stability training increases the ability to stabilise the body during movement by simultaneous contraction of the muscles of the abdominal wall, the pelvis, the lower back and the diaphragm. Examples of core stability training are Pilates, training with an exercise ball (Swiss ball) and bridges. For a long time it was thought that exercise training, both

in Pompe disease as well as in other myopathies, should focus on endurance and not on resistance in order to improve the muscle condition without causing even more muscle damage.[86-88]

Studies so far have highlighted that findings obtained by exercise studies cannot automatically be translated to other all myopathies, since the training outcome and possible negative and positive effects may vary. For example, muscular dystrophies which are characterized by the lack of a key protein that is needed to maintain the integrity and proper functioning of the muscle may benefit from other exercise regimens than metabolic myopathies including mitochondrial disorders, in which there is a defect in the energy metabolism of the muscle, or inflammatory myopathies in which muscle weakness is caused by muscle inflammation. Therefore, the outcome of high-resistance training most probably differs between disorders caused by abnormalities of structural proteins and those in which the morphology is still intact.

When it comes to Pompe disease few studies had been conducted on exercise training so far. The largest was performed before ERT became available and was published in 2007.[89] For a mean duration of four years, the 26 patients included in it participated in a combined nutrition and endurance exercise therapy program that led to improved muscle function as measured with the Walton score. In 2011, a study was published in which 5 Pompe patients followed a 20-week aerobic-resistance training program combined with ERT.[90] At a group level, muscular strength and walking distance both improved. Although these studies suggested that exercise training could be beneficial, additional evidence would make a stronger case. Table 1 gives an overview of the clinical studies on the effects of exercise training in other types of myopathies besides Pompe disease.[90-106] Only studies with ≥5 patients were included.

Although some case reports reported muscle weakness caused by overwork as site effect,[107,108] the larger studies presented in Table 1 do not. Despite the use of different training regimens improvement in at least one outcome measure was obtained in all studies. This suggests that exercise training elicits positive effects in a variety of myopathies.

AIMS AND OUTLINE OF THIS THESIS

This thesis encompasses a number of studies aimed at gaining insight in three different aspects of Pompe disease where evidence was still lacking. These aspects include the role of muscle fiber type differences in the pathogenesis of Pompe disease; the consequences of muscle weakness on the body composition and bone mineral density; and the role of exercise training for improving the patients' functioning.

After a general introduction on Pompe disease (**Chapter 1**), **Chapter 2** presents a case of adult onset Pompe disease with a rather unusual clinical presentation and a rather extreme fiber-type-specific lysosomal glycogen storage within type 1 fibers, while type 2 fibers were spared. This case led us to study the muscle fiber-type distribution and the muscle fiber-type-specific damage in quite some detail in a group of 22 patients (**Chapter 3**). **Chapter 4** and **5** describe the bone mineral density and the body composition of patients with Pompe disease. First, candidate causes of decreased bone mineral density were identified in Pompe patients during their natural course **(Chapter 4).** This study was followed by a study on the effects of ERT on bone mineral density and body composition **(Chapter 5)**. **Chapter 6** and **7** report on the feasibility and safety performing a standardized and well-structured exercise intervention program combining aerobic, resistance and core stability exercises in combination with enzyme replacement therapy. **Chapter 6** focuses on endurance, muscle strength, muscle function and core stability while the focus in **Chapter 7** is on fatigue, pain, mental health and physical functioning, as assessed by patients reported outcomes. **Chapter 8, the General Discussion,** provides an overview of the results and future perspectives.

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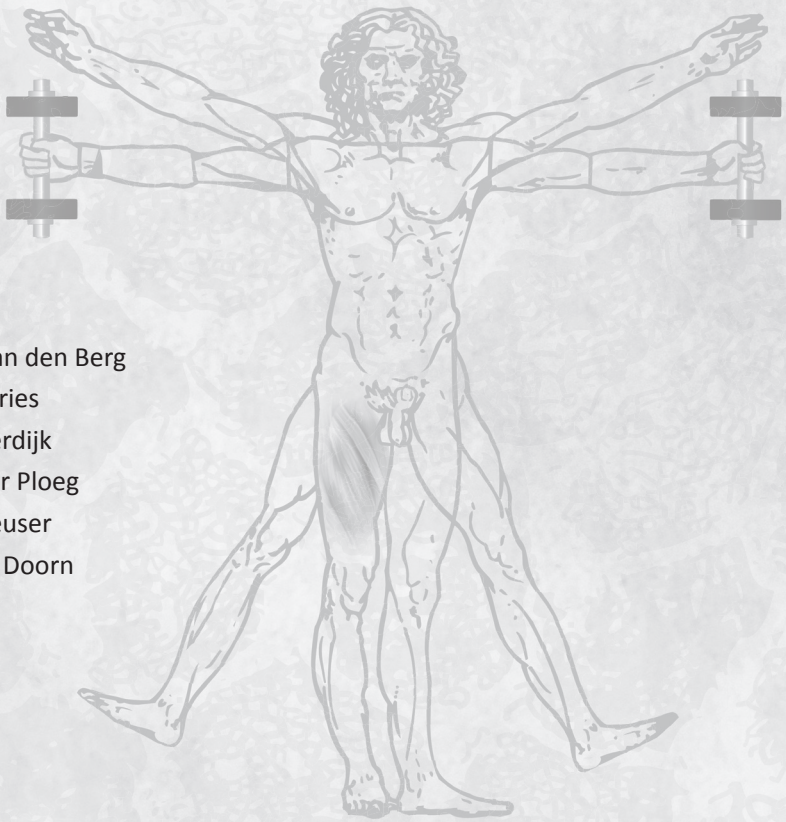
CHAPTER 2

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 (ERT) 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. 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. Representative 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.

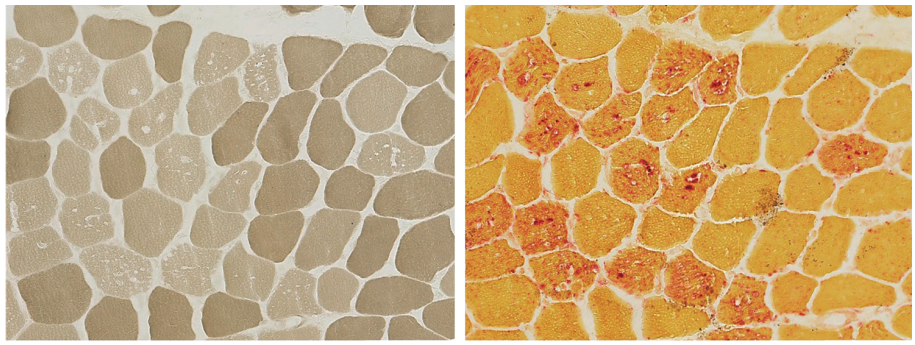

Figure 1 | Biopsy from the m.quadriceps femoris showing selective involvement of type 1 muscle fibers. ATPase staining at pH 9.4: Type 1 muscle fibers are lightly stained and are vacuolated (panel A). Acid phosphatase staining (red) of a serial section demonstrates lysosomal pathology in the type 1 muscle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

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 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 ERT.

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 ERT 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 and to identify the biochemical and biological basis possibly underlying such differences.

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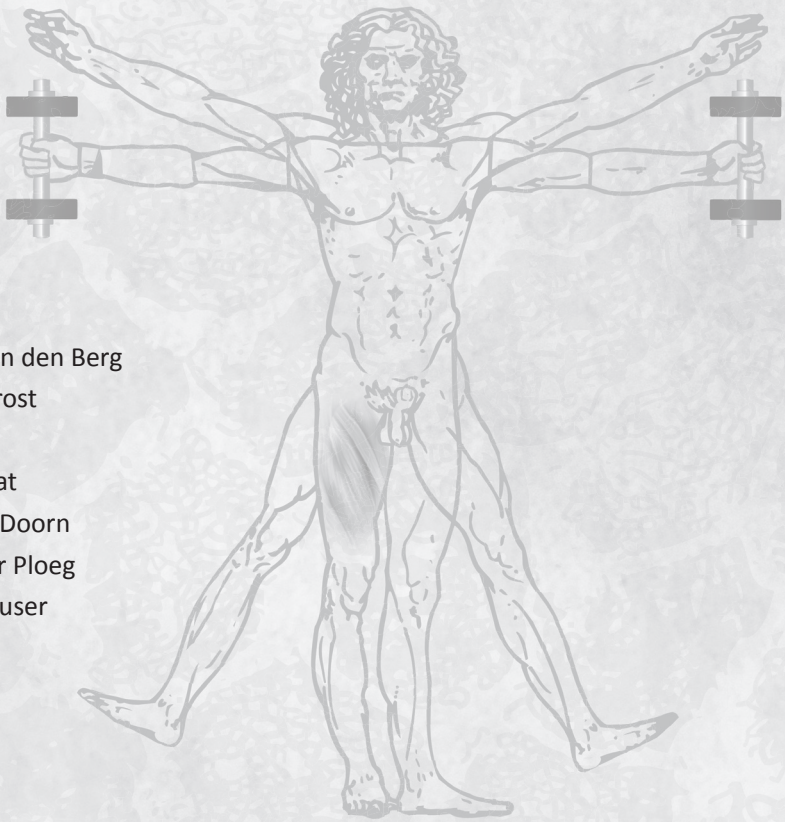
CHAPTER 3

Muscle fiber-type distribution, fiber-type-specific damage, and the Pompe disease phenotype



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ABSTRACT

Pompe disease is a lysosomal storage disorder caused by acid α-glucosidase deficiency and characterized by progressive muscle weakness. Enzyme replacement therapy (ERT) has ameliorated the patients' perspectives, but the reversal of skeletal muscle pathology remains a challenge. We have studied pretreatment biopsies of 22 patients with different phenotypes to investigate to what extent fiber-type distribution and fiber-type-specific damage contribute to the clinical diversity.

Pompe patients have the same fiber type distribution as healthy persons, but among non-classic patients with the same *GAA* mutation (c.-32-13T>G) those with early onset of symptoms tend to have more type 2 muscle fibers than those with late-onset disease. Further, it seemed that the older, more severely affected classic infantile patients and the wheelchair-bound and ventilated non-classic patients had a greater proportion of type 2x muscle fibers. However, like in other diseases this may be caused by physical inactivity of those patients.

INTRODUCTION

Pompe disease (glycogen storage disease type II, acid maltase deficiency) (OMIM 232300) is an inherited lysosomal storage disorder caused by the deficiency of acid α-glucosidase (EC 3.2.1.3). Deficiency of this lysosomal enzyme leads to glycogen accumulation in a variety of tissues, including skeletal muscle.[1,2] Pompe disease shows a broad clinical spectrum, ranging from the classic infantile form characterized by hypotonia, hypertrophic cardiomyopathy and death within the first year of life[3,4] to more slowly progressive forms characterized by proximal muscle weakness and respiratory problems in children and adults.[5,6]

Skeletal muscle weakness is attributed to the loss of mechanical force through deposition of the glycogen-loaded lysosomes between the contractile myofibrils followed by muscle damage by lysosomal rupture and release of lysosomal enzymes into the cytoplasm.[7-9] A recent study in infants with Pompe disease confirmed this view.[10] In mice with Pompe disease and in affected adults the accumulation of autophagic debris seems to aggravate the skeletal muscle damage.[10-12] Unlike in mice, the autophagic accumulation in humans is not restricted to type 2 muscle fibers but also involves type 1 fibers.[10-12]

The introduction of enzyme replacement therapy (ERT) in 2006 has changed the perspectives of patients with Pompe disease.[2,13-17] ERT has largely solved the cardiac problem, but the reversal of skeletal muscle pathology remains a challenge.[18] In a recent case report, we drew attention to the occurrence of fiber-type-specific pathology and its potential relevance for clinical presentation and responsiveness to ERT.[19] In the study reported here, we assessed 22 patients with Pompe disease with regard to fiber-type distribution and fiber-type-specific damage in order to investigate to what extent this might contribute to the clinical diversity.

METHODS

This study was performed at the Erasmus MC University Medical Center in Rotterdam, The Netherlands, in collaboration with the Maastricht University, Maastricht, The Netherlands. The Ethical Committee of the Erasmus MC University Medical Center has approved the research protocol. Written informed consent was obtained from all patients or their parents. Twenty-two patients were selected for this study, representing a cross-section of the Dutch Pompe patient population and divided over different groups (classic versus non-classic Pompe disease, and the latter group subdivided in clearly distinct ages of onset; <15 years or >30

years). Diagnosis was in all cases established by clinical and biochemical assessments. Muscle biopsies, either open surgical biopsies or needle biopsies from the m. vastus lateralis, were taken just before the start of ERT.

Immunohistochemistry

Muscle biopsies were embedded in Tissue-Tek (Aurian, Wageningen, The Netherlands) and immediately frozen in melting isopentane. All tissue samples were stored at -80°C until analysis.

Immuno-staining was performed on cryostat sections (5 µm) To identify muscle fiber types we used two different staining procedures. The first identifies type 1 and type 2a muscle fibers in combination with laminin. For this staining we used the following primary monoclonal mouse antibodies: A4.840, anti-human myosin heavy chain (MHC) type 1 [Developmental Studies Hybridoma Bank (DSHB), Iowa City, Iowa]; N2.261, anti-human MHC type 2a (DSHB); and L-9393, anti-human laminin (Sigma, Zwijndrecht, The Netherlands). The second staining procedure identifies type 2x muscle fibers as opposed to type 1 plus type 2a fibers, and laminin. The following primary antibodies were used for this staining: N2.261, anti-human MHC type 1 and type 2a (DSHB); 6H1, anti-human MHC type 2x (DSHB); and L-9393, anti-human laminin (Sigma, Zwijndrecht, The Netherlands). After incubation with the primary antibodies, the sections were incubated with a mixture of appropriate conjugates, i.e. goat anti-mouse IgM Alexa-Fluor 555, goat anti-mouse IgG1 Alexa-Fluor 488, and goat anti-mouse IgG Alexa-Fluor 350. Images were obtained using a Nikon E800 fluorescence microscope (Nikon, Amsterdam, The Netherlands) coupled to a progressive scan color CCD camera (Basler 101C).

Analysis of stained tissue sections

To obtain a representative overview of a stained muscle section, ten images were taken at separate locations of a section using a 20x objective lens. This procedure enabled analysis of at least 25 fibers per image. The number of each of the different muscle fiber types was counted and the total number of type 2 muscle fibers was taken as the sum of type 2a and type 2x muscle fibers. The percentage surface area occupied by vacuoles was estimated with a planimetric method. The degree of muscle fiber vacuolation was scored in four categories: very severe: 100%-75% vacuolated; severe: 74%-50% vacuolated; intermediate: 49%-25% vacuolated; mild or normal: 24%-0% vacuolated. Muscle fibers with >25% of vacuoles were considered as damaged fibers. The total percentage of damaged muscle fibers was calculated as well as the percentage of damaged fibers per muscle fiber type.

Clinical parameters

Laboratory analysis

We analyzed non-fasting blood samples for serum concentrations of Creatine Kinase (normal values <295 U/L in infants; <230 U/L in young children; <270 U/L in teenage boys and <123 U/L in teenage girls; <200 U/L in men and <170 U/L in women).

Pulmonary function

Pulmonary function tests were performed in all patients >4 years. Pulmonary function was measured with spirometry in all patients while sitting, and in non-ventilated patients also in supine position. Historical data were used for comparison.

Muscle strength

The muscle strength measurements were performed in all patients >6 years, being measured in 12 muscle groups with a hand-held dynamometer (dynamometer type CT 3001-C.I.T. Technics). Maximum contraction values were assessed with the break technique in which the examiner applies adequate force to overcome the examinee, thereby producing an eccentric contraction. The values obtained for the different muscle groups were expressed as percentages of age- and sex-matched reference values. We used summed scores for total muscle force (neck flexors, neck extensors, shoulder abductors, elbow flexors, elbow extensors, wrist extensors, hip flexors, hip abductors, knee flexors, knee extensors, foot dorsiflexors, and foot plantar flexors) and proximal muscle force (shoulder abductors, elbow flexors, and extensors, hip flexors and hip abductors, knee flexors, and extensors). All muscle groups were assigned equal weight. Isometric muscle strength of the quadriceps femoris muscle was measured by quantitative muscle testing (QMT) on a Biodex® isokinetic dynamometer (Model 2000, Multijoint System 3, Biodex Corporation, Shirley, NY, USA). Values obtained per group are expressed as percentages of age- and sex-matched reference values as provided by the manufacturer.

Fatigue Assessment

This assessment was obtained in all adult patients. The severity and impact of fatigue was assessed using the Fatigue Severity Scale (FSS).[20] The total FSS score is the average of the nine-item scores and ranges from 1 (no signs of fatigue) to 7 (most disabling fatigue).

Statistical analysis

Descriptive statistics are presented as mean ± standard deviation. Student's *t*-test was used for the comparison of the means, after validating normality assumptions. Nonparametric tests for independent samples (Mann-Whitney and Kruskal-Wallis tests) were used for the other variables. Pearson's correlation coefficients were calculated in order to ascertain the relationships between the percentage of damaged muscle fibers and age, disease duration at time of the biopsy, and clinical parameters. All analyses were performed using SPSS for Windows (version 15.0, SPSS Inc. Chicago, IL, USA). Two-sided *P*-values <0.05 were considered significant.

RESULTS

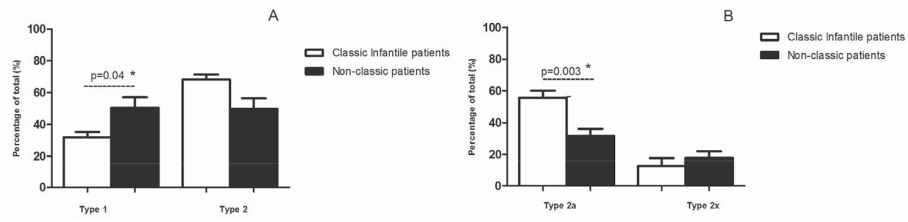
Patients

Muscle biopsies of 22 patients were selected for this study. Nine patients had the classic infantile form of Pompe disease (group 1), and 13 were non-classic patients. Four of the latter 13 had their first symptoms before the age of 15 years (group 2). The other nine had their first symptoms after the age of 30 years; four of them were severely affected (group 3) and five were mildly affected (group 4) at the time that the biopsy was taken. None of the patients had ever received ERT. Supplementary Table 1 summarizes the characteristics of the patients.

Muscle fiber typing

Classic infantile patients compared with non-classic patients had a lower percentage of type 1 fibers (P=0.04) and a significantly higher percentage of type 2a muscle fibers (P<0.01) (Supplementary Figure 1). The latter difference is mainly due to the difference between infants (group 1) and adults >age of 30 (groups 3 and 4) (Figure 1).

Figure 2 shows correlations between patients´ ages and muscle-fiber types. For the total group, a significant decrease of type 2a muscle fibers was found with increasing age (Figure 2c, d; Spearman Rho -0.703, P<0.001). In classic infantile Pompe patients, the decrease in the percentage of type 2a muscle fibers (Spearman Rho -0.773, P=0.015) coincided with a significant increase in type 2x muscle fibers (Figure 2C, E; Spearman Rho 0.950, P<0.001).



Supplementary Figure 1 | The percentage of type 1 and type 2 muscle fibers (panel A), and the percentage of type 2a and type 2x (panel B) muscle fibers in classic infantile patients and non-classic patients. Significance between the different groups of patients is shown by * (*P*<0.05).

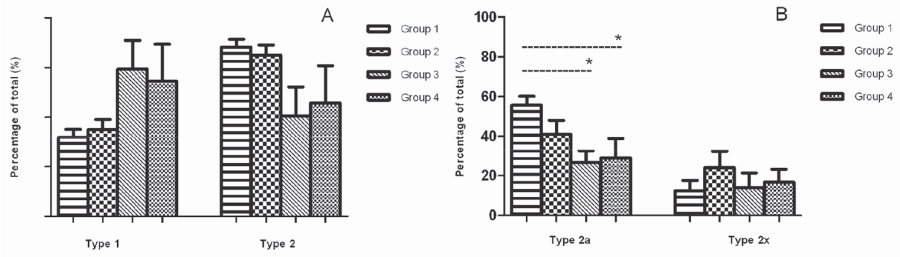


Figure 1 A | Percentage of type 1 and type 2 muscle fibers and B. type 2a and type 2x muscle fibers in classic infantile patients (group 1), patients with the *IVS1* mutation and symptom onset before the age of 15 years (group 2), and mildly and severely affected patients with the *IVS1* mutation and symptom after the age of 30 years (groups 3 and 4, respectively). B Group 1: 55.8%±9.8 (37-73%), group 3 29.0%±20.2 (10-47%) and group 4: 26.6%±13.7 (3-39%). Significance between the different groups of patients is shown by * (*P*<0.05).

No significant differences in muscle-fiber-type distribution were found between ambulant and wheelchair-bound patients (Figure 3A) or between ventilated and non-ventilated patients (Figure 3B). However, wheelchair-bound patients seemed to have a slightly higher percentage of type 2x muscle fibers than did ambulant patients. Also, ventilated patients seemed to have a slightly higher percentage of type 2x muscle fibers than did non-ventilated patients.

Muscle fiber damage

The left column of Table 1 shows that the percentage of muscle fibers with >25% vacuolation (damaged fibers) did not differ between the four subgroups of Pompe patients. The three columns at the right illustrate the percentage of damaged muscle fibers per muscle fiber

type. Damage of type 1 muscle fibers was more prominent in classic infantile Pompe disease than in non-classic disease (*P*=0.04). With regard to the degree of vacuolation, there were no significant differences between the four subgroups (Supplementary data, Table 2).

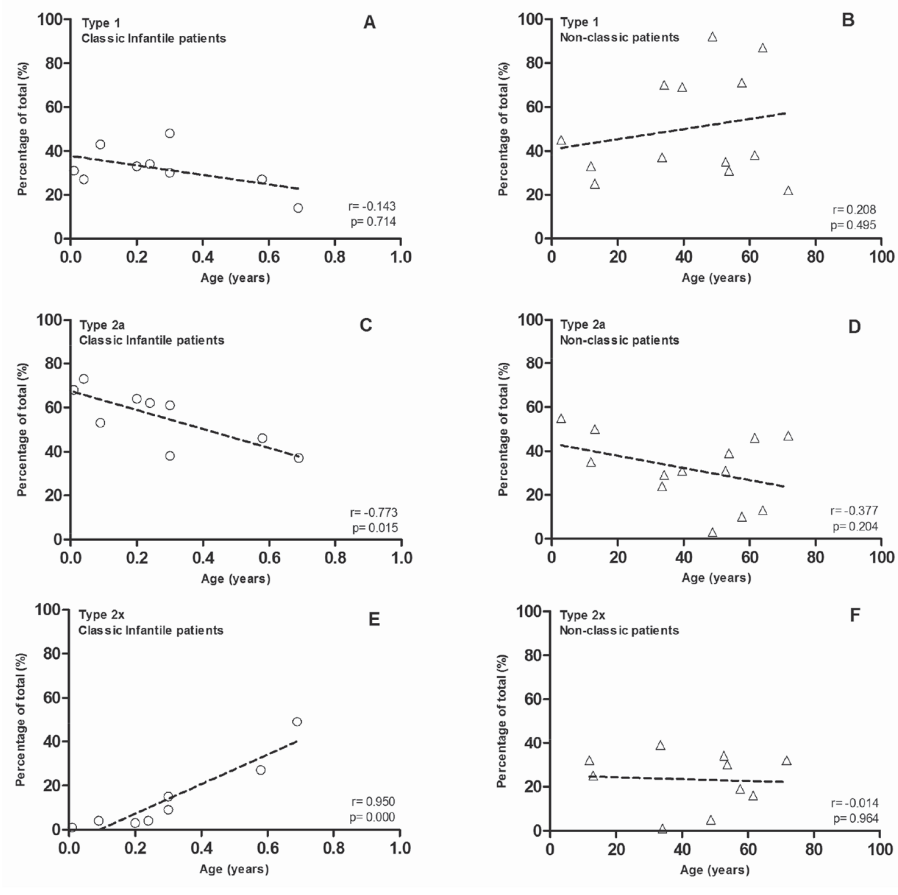


Figure 2 A, B | Type 1 muscle fibers, C, D type 2a and E, F type 2x versus age in classic infantile patients and patients with the *IVS1* mutation. Correlations are significant (*P*<0.05) for age and type 2a muscle fibers in classic infantile patients (C Pearson's *r*=-0.773) and for age and type 2x muscle fibers in classic infantile patients (E Pearson's *r*=0.950)

Table 1 | Percentage of damaged muscle fibers and damaged fibers for each fiber type

	Damaged muscle fibers (%)*	Damaged fibers per muscle fiber type (%)		
		Type1	Type 2a	Type 2x
Classic infantile patients	66.3±21.8 (32-97)	78.0±24.9 (20-100)	50.0±32.7 (7-92)	67.4±37.9 (0-100)
Non-classic patients	62.8±21.8 (47-97)	49.9±24.2 (0-98)	74.2±18.8 (42-100)	53.6±29.6 (4-100)
First symptoms <15 years	62.8±21.3 (47-94)	45.0±50.8 (0-98)	81.5±16.0 (58-94)	35.3±37.6 (4-77)
First symptoms >30years; mildly affected	60.2±10.3 (49-70)	47.5±17.2 (19-63)	72.4±21.3 (42-100)	69.5±25.2 (40-100)
First symptoms >30 years; severely affected	66.0±22.8 (47-97)	57.8±27.2 (28-93)	69.3±20.9 (51-99)	50.7±23.7 (31-77)

Mean ± standard deviation (range). * Damaged muscle fibers are the fibers with more than 25% vacuolation.

Supplementary Table 2 | Degree of vacuolation per muscle fiber type (percentage of fibers)

	Very severe (100-75% vacuolation)	Severe (74-50% vacuolation)	Intermediate (49-25% vacuolation)	Mild or normal (24-0% vacuolation)
Type 1	10.5%±21.2 (0-71%)	14.5%±10.3 (0-34%)	36.5%± 24.9 (0-75%)	38.6%±31.3 (0-100%)
Type 2a	11.2%±15.6 (0-65%)	16.3%±11.8 (0-42%)	36.8%±18.3 (3-67%)	35.6%±27.5 (0-92%)
Type 2x	17.6%±29.6 (0-100%)	17.2%±24.8 (0-100%)	24.9%±20.0 (0-59%)	40.3%±33.9 (0-100%)

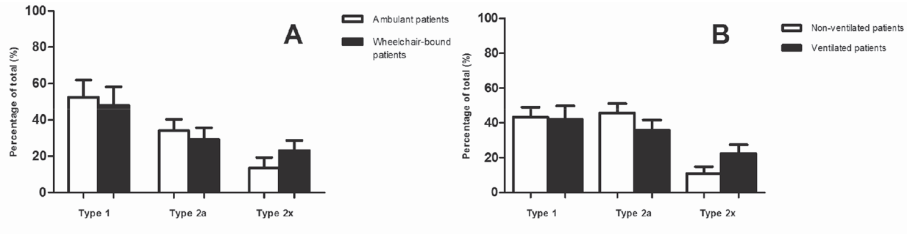


Figure 3 | Differences in muscle-fiber-type distribution between A ambulant and wheelchair-bound patients and B ventilated and non-ventilated patients.

Although we did not find statistical differences in the percentage of damaged fibers or in the degree of vacuolation between subgroups, muscle biopsies of mildly (Figure 4, left panels) and severely (Figure 4, right panels) affected patients had a quite different appearance. Biopsies of the mildly affected patients showed normally shaped and neatly organized muscle fibers, whereas biopsies of the severely affected patients showed irregularly shaped and loosely organized muscle fibers.

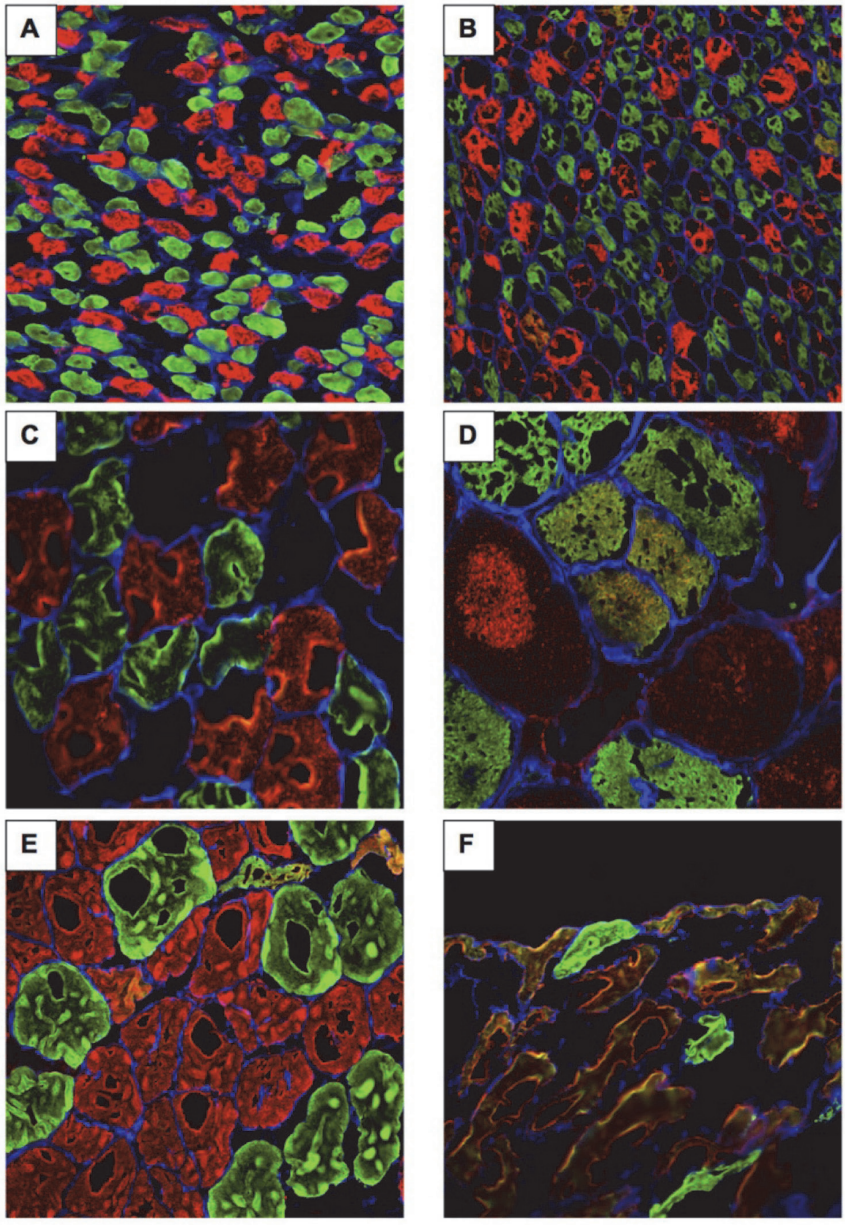


Figure 4 | Differences in appearance of muscle fibers in A classic infantile patient with an early diagnosis b classic infantile patient with a late diagnosis, C mildly and D severely affected patient with the *IVS1* mutation and symptom onset before 15 years of age; E mildly and F severely affected patient with the *IVS1* mutation and symptom onset after 30 years of age. Fiber typing was performed with antibodies against myosin heavy-chain (MHC) type 1 (*red*) and MHC type 2a (*green*) fibers. MHC-negative regions within muscle fibers indicate the presence of vacuoles. Anti-laminin staining (*blue*) was used to mark fiber boundaries.

Muscle fiber damage and clinical parameters

Figure 5 shows correlations between percentage of damaged muscle fibers and the different clinical parameters. We found significant correlations between the percentage of damaged muscle fibers and (1) forced vital capacity (FVC) in the supine position (r=-0.862, P=0.027), (2) proximal muscle strength tested by hand-held dynamometry (r=-0.762, P=0.046), and (3) quadriceps femoris muscle strength by QMT (r=-0.744, P=0.022). The correlation between the percentage of damaged muscle fibers and the total muscle strength by hand-held dynamometry was close to significant (r=-0.748, P=0.053). Four patients were excluded from the analysis for muscle strength by hand-held dynamometry and two from the analysis for muscle strength of the quadriceps femoris muscle by QMT because data could not be obtained for some muscle groups.

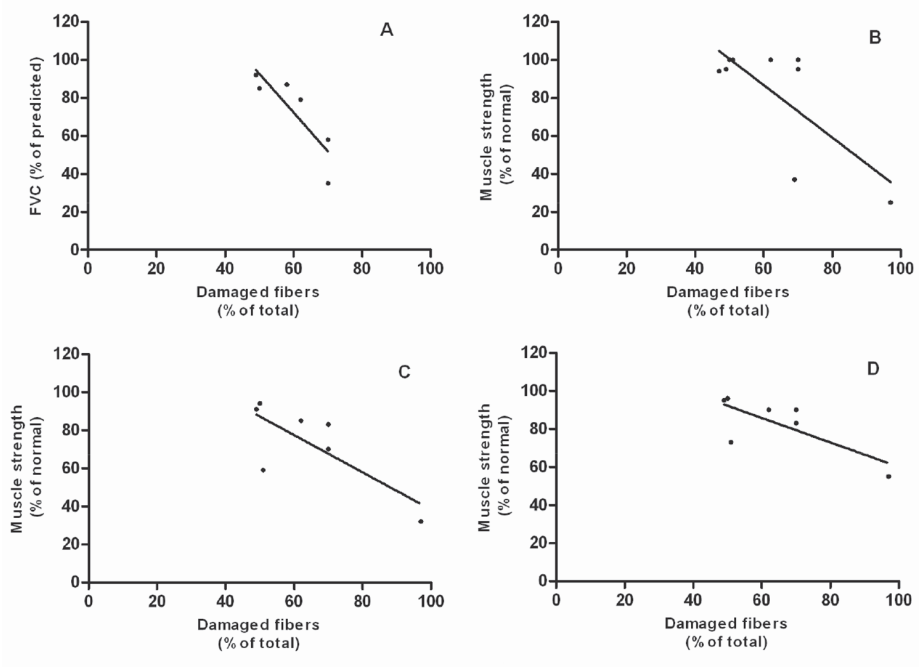


Figure 5 | A Forced vital capacity (FVC) in the supine position, B muscle strength of the quadriceps femoris, C muscle strength of the proximal muscles, and D total muscle strength versus percentage of damaged muscle fibers. Correlations are significant (P<0.05) for FVC in supine position (A Pearson's r=-0.862), muscle strength of the quadriceps femoris (B Pearson's r=-0.744), and muscle strength of the proximal muscles (C Pearson's r=-0.862). Total muscle strength showed a trend (D Pearson's r=-0.748).

Severely affected non-classic patients seemed to have lower levels of serum CK than patients in the other groups of (279.8±125.5 U/L in severely affected patients with onset >30 years vs 894.4±641.3 U/L in classic infantile patients, 720.0±456.8 U/L in patients with symptom onset <15 years of age, and 948.8±530.1 U/L in mildly affected patients with symptom onset >30 years). There were no significant correlations between the percentage of damaged muscle fibers and FVC in sitting position and FSS.

DISCUSSION

ERT has ameliorated the perspectives of patients with Pompe disease. Knowledge of prognostic factors as well as factors predicting the response to ERT has become increasingly important. The aims of our present study were to investigate to what extent skeletal muscle fiber-type distribution and skeletal muscle-fiber type specific damage contribute to the clinical course of Pompe disease.

Skeletal muscle fiber-type distribution

Compared with non-classic forms of Pompe disease, classic infantile patients had a lower percentage of type 1 and a higher percentage of type 2a muscle fibers. As little is known about muscle fiber-type distribution in healthy infants, it is hard to say whether this finding is physiological or related to Pompe disease pathophysiology. Healthy 6- to 50-year-old humans have an equal distribution of type 1 and type 2 fibers in the vastus lateralis, although the range is very wide (from 20-75% type 1 muscle fibers).[21,22] Skeletal muscle fiber-type distribution in non-classic patients appeared to be no different among the selected subgroups.

Skeletal muscle fiber-type distribution – The effect of aging

In healthy humans, skeletal muscle aging atrophy starts at the age of 25 years and accelerates thereafter.[22] Aging atrophy is due to a reduction in both the number and the size of mainly type 2 muscle fibers.[23-25] In patients with Pompe disease, we observed the same age-related effect. Interestingly, in classic infantile Pompe disease, a loss of type 2a muscle fibers was observed with increasing age coinciding with a gain of type 2x muscle fibers. Though this could be a physiological phenomenon,[26] it could also be due to immobility of the older, more severely affected infants in our study group. Also, the non-ambulant adults had a relatively

high percentage of type 2x muscle fibers. As known from other diseases, reduced physical activity can alter fiber type composition towards a greater proportion of type 2x fibers.[27]

Skeletal muscle fiber damage

In classic infantile Pompe patients, muscle pathology closely parallels clinical findings and clinical response to ERT.[9,28] In non-classic patients, the diagnostic value of muscle biopsies is rather limited, and some biopsies may not even show a sign of glycogen storage.[18,29-31] In our study, we did see skeletal muscle damage in all muscle biopsies. We quantified the muscle damage by using the percentage of damaged muscle fibers and the degree of muscle fiber vacuolation but found no significant differences between the four subgroups, which confirms earlier findings.[29] Nevertheless, muscle biopsies of mildly and severely affected patients in our study had a quite different appearance. Shoser et al. observed more pronounced secondary alterations in severely affected patients.[29] This may stress the importance of a more descriptive qualification, instead of quantification, of muscle damage.

Skeletal muscle fiber damage and the clinical picture

CK is a widely used marker in muscle disease, and the degree of CK elevation reflects the underlying disease process. In chronic myopathies, the CK level can drop back to normal due to loss of muscle mass.[32,33] Similarly, we found in this study that severely affected adult Pompe patients had near normal CK levels whereas less affected adults had higher levels. With respect to other clinical parameters, we found a clear correlation between the percentage of damaged muscle fibers and the loss of proximal muscle strength, as well as a concordant loss of pulmonary function in supine position.

Potential relevance of our findings for therapeutic outcome

Based on what is known from the literature, the greater proportion of type 2x muscle fibers in severely affected infants and adults in our study, together with the more severe vacuolation of these fibers, may hamper the effectiveness of ERT, as type 1 fibers were reported to respond better to ERT than were type 2, 2b fibers in particular.[34-36] This also supports the notion that early treatment leads to better outcome.[15]

In summary, our study demonstrates that muscle fiber-type distribution in Pompe patients does not differ from that in healthy persons. Furthermore, it seemed that the older, more severely, affected classic infantile patients and the wheelchair-bound and ventilated non-

classic patients had a greater proportion of type 2x muscle fibers. However, this may be caused by physical inactivity in those patients, which is also seen in other diseases.

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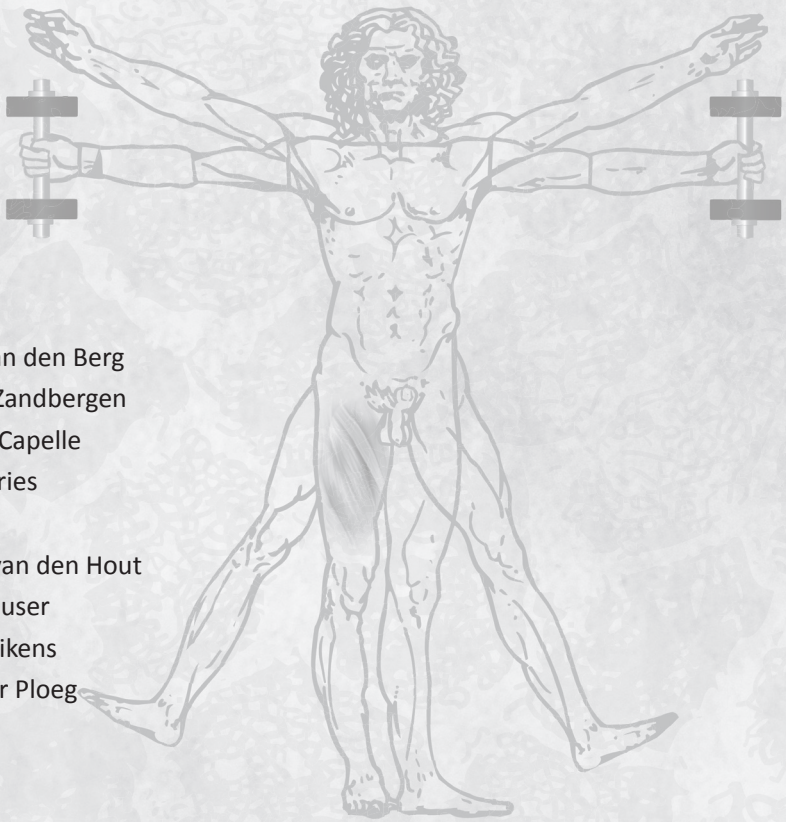
CHAPTER 4

Low bone mass in Pompe disease muscular strength as a predictor of bone mineral density



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ABSTRACT

Pompe disease is an inherited metabolic myopathy caused by deficiency of acid alpha-glucosidase. The introduction of enzyme replacement therapy as treatment for the disease may change prospects for patients and may require that more attention be paid to co-morbidities such as osteoporosis.

Methods: Bone mineral status was assessed in children and adults with Pompe disease and compared with reference values by means of dual energy X-ray absorptiometry (DXA) technology (GE Lunar DPX, GE Health Care). Bone mineral density (BMD) of the total body and the lumbar spine (L2-L4) was measured in adults and children;BMD of the femoral neck was measured in adults only. Exclusion criteria were: age <4 years, severe contractures, and inability to transfer the patient.

Results: 46 patients were enrolled in de study; 36 adults and 10 children. The BMD was significantly lower in Pompe patients than in healthy individuals. Sixty-seven percent of patients had a decreased BMD *Z*-score below -1; 26% were classified as osteoporosis/low bone mass for chronological age (T-score<-2.5 in adults or *Z*-score<-2 in children, 66% had a BMD *Z*-score below -1 of the femoral neck and 34% had a BMD *Z*-score below -1 for the lumbar spine. Osteoporosis/low bone mass for chronological age was more frequent in patients who were wheelchair-bound, but was also observed in ambulant patients. We found a significant correlation between proximal muscle strength and total body BMD. Of the 10 children, 8 (all four patients with the classic infantile form) had a low BMD.

Conclusion: Low BMD is a frequent finding in patients with Pompe disease and may be causally related to decreased proximal muscle strength. BMD should be monitored at regular intervals. Children deserve specific attention.

INTRODUCTION

Pompe disease (glycogen storage disease type II, acid maltase deficiency) (OMIM 232300) is an inherited metabolic myopathy caused by deficiency of acid α-glucosidase.[1] Deficiency of this lysosomal enzyme leads to glycogen accumulation in a variety of tissues. The *GAA* gene is located on chromosome 17q25.3. More than 200 mutations have been detected in the *GAA* gene.[1,2] Pompe disease presents as a broad clinical spectrum, ranging from the classic infantile form characterized by hypotonia, hypertrophic cardiomyopathy and death within the first year of life[3,4] to more slowly progressive forms characterized by proximal muscle weakness and respiratory problems in children and adults.[5,6] The most common genotype in children and adults with Pompe disease is the IVS1 c.-32-13T>G/null genotype.[7,8]

Though the prognosis of patients is poor, this may change with the introduction of enzyme replacement therapy (ERT). The therapy has led to increased survival and improved motor outcome in patients with the classic infantile form.[9-11] Data on ERT in children and adults are still limited, but early results indicate that muscle and respiratory function may stabilize or improve.[12,13] These potential changes in outcome imply that better long-term clinical management programs will be needed for infants, children and adults with Pompe disease. These programs should also focus on co-morbidities.

Decreased bone mineral density (BMD) and increased incidence of fractures have been observed in several myopathies[14-16] and lysosomal storage diseases.[17,18] It seems likely that patients with Pompe disease are at a greater risk acquiring osteoporosis – a skeletal disorder characterized by comprised bone strength predisposing a person to an increased risk of fracture.[19] There is evidence to suggest that bone fractures occur more frequently in infants and children with Pompe disease, especially in those who are immobile and bedridden.[20] However, no BMD data are available for child, adolescent, or adult Pompe disease patients. The goal of this study was therefore to systematically assess the bone mineral status in a cohort of forty-six patients with Pompe disease (children, adolescents and adults) via dual-energy X-ray absorptiometry (DXA) technology, to ascertain the prevalence of osteoporosis/low bone mass for chronological age and to identify candidate causes of possible decreased BMD in this patient group.

PATIENTS AND METHODS

This study on the bone mineral status was performed at the Erasmus MC University Medical Center in Rotterdam, The Netherlands, which serves as the Dutch national referral center and international expert center for patients with Pompe disease. The Ethical Committee of the Erasmus MC University Medical Center approved the research protocol. Written informed consent was obtained from all patients or their parents.

Exclusion criteria comprised age <4 years, severe contractures and inability to transfer the patient to the DXA table.

Forty-six patients were enrolled in this project since 1995. The diagnosis of Pompe disease was confirmed in all patients through mutation analysis and measurement of decreased acid α-glucosidase activity in leukocytes or fibroblasts. The distinction between classic infantile and milder variants with Pompe disease is made on the basis of clinical presentation, residual alpha-glucosidase activity, and severity of the mutation in the *GAA* gene.

The DXA scan was performed on the adults just before or within 34 weeks after start of ERT (mean 11 weeks; range 0-34 weeks). At the time of their DXA scan, all but one of the children/ adolescents had been undergoing enzyme replacement therapy for longer (mean 3.7 years; range 0-8 years).

Anthropometrics

Body weight with indoor clothing without shoes was measured on a digital scale to the nearest 0.1 kg (ServoBalans type KA-20-150S, Servo Berkel Prior B.V.). The height of ambulant patients was measured barefoot standing (Ulmer Stadiometer, Prof. E. Heinze); wheelchair-dependent patients were measured while lying in bed. The Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Bone densitometry scanning

Bone densitometry was performed by using DXA technology (GE Lunar DPX, GE Health Care). In adults, the BMD of the total body, the lumbar spine (L2-L4), and femoral neck was measured. In children/ adolescents (age ≤20 years) only the BMD of the total body and the lumbar spine (L2-L4) were measured, because reference values were available from a study of 444 healthy white volunteers aged between 4 and 20 years in the Netherlands.[21] The BMD results were expressed as *Z*-scores, which are the number of standard deviations above or below the mean reference values for healthy persons matched for age, sex, length, weight and ethnic group.

To identify osteoporosis in adult, the *T*-score, was used. A *T*-score for total body, lumbar spine or femoral neck of less than or equal to -2.5 SD was defined as indicating osteoporosis. In children/adolescents the term "low bone mass for chronological age" is preferred when the *Z*-score is less than or equal to -2 SD.[22]

The reference values used in the DPX software are from studies of the reference populations in university medical centers and clinics in the US, England and Northern Europe.

Daily quality assurance tests were performed with a calibration block supplied by the manufacturer. Repeated measurements on the calibration block had coefficients of variation <0.5%. In addition, a calibration aluminium phantom was measured weekly; the coefficients of variation were <0.5%.

Laboratory analysis

We analyzed serum concentrations of calcium (normal values; 2.10 to 2.60 mmol/L in children and 2.20 to 2.65 mmol/L in adults) and phosphate (normal values; 1.00 to 1.80 mmol/L in children and 0.80 to 1.40 mmol/L in adults), 25-hydroxyvitamin D (normal values; 50-136 nmol/L), and parathyroid hormone (normal values; 1.4 to 7.3 pmol/L). As markers of bone turnover we assessed the bone formation marker alkaline phosphatase and the bone resorption marker β-CTx (normal values are shown in Table 4). β-CTx concentrations were determined using an electrochemiluminescence immunoassay (ECLIA) following the manufacturer's instructions (β-CrossLaps/serum, Cobas®, Roche Diagnostics, Mannheim, Germany).

Lung function

Pulmonary function (FVC) was measured with spirometry. Historical data were used for comparison.

Muscle strength

The muscle strength of twelve muscle groups was measured in Newtons with a hand-held dynamometer (dynamometer type CT 3001-C.I.T. Technics). Maximum isometric contraction values were assessed with the break technique, in which the examiner applies adequate force to overcome the examinee, thereby producing an eccentric contraction. The values obtained for the different muscle groups were expressed as percentages of age- and sex-matched reference values. We used summed scores for total muscle force (neck flexors, neck extensors, shoulder abductors, elbow flexors, elbow extensors, wrist extensors, three-point grip, hip flexors, hip abductors, knee flexors, knee extensors, foot dorsiflexors and foot plantiflexors),

muscle force of the neck region (neck flexion and extension), proximal muscle force of the upper extremity (shoulder abductors, elbow flexors and extensors), distal muscle force of the upper extremity (wrist extensors), proximal muscle force of the lower extremity (hip flexors and abductors, knee flexors and extensors) and distal muscle force of the lower extremity (foot dorsiflexors and plantiflexors). All muscle groups were assigned equal weight. The values obtained for children could not be incorporated in the analysis since insufficient age-matched reference values were available for the various muscle groups.[23]

Statistical analysis

Descriptive statistics are presented as mean ± SD. All variables were analyzed to evaluate their normality (Shapiro-Wilk test), and then the appropriate statistical tests were chosen. Student's *t*-test was used for the comparison of the means, after validating the normality assumptions. The nonparametric tests for independent samples (Mann-Whitney test and Kruskal-Wallis test) were used for the other variables.

Pearson correlation coefficients were calculated in order to ascertain the relationships between muscle strength and BMD. Multiple regression analysis was used to evaluate various factors simultaneously in relation to BMD *Z*-scores. All the analyses were performed using SPSS for Windows (version 15.0, SPSS Inc. Chigago, Il., USA). Two-sided *P*-values less than 0.05 were considered significant.

RESULTS

Patients

Forty-six patients (23 males, 23 females) were enrolled in this study. Thirty-six were adults and ten were children/adolescents. The most common genotype c.-32-13T>G (IVS1-13T>G) mutation was present in 84% of the patients. The patient characteristics are summarized in Table 1.

DXA scanning

Table 2 shows the BMD *Z*-scores of the children/adolescents and adults compared with the reference values of healthy individuals. The *Z*-scores for total body and femoral neck were significantly lower in the Pompe patients. The mean *Z*-scores of lumbar spine were not significantly different from zero. The patients' TB and FN BMD scores are plotted against reference values for healthy individuals in Figure 1.

Table 1 | Patient characteristics

	Sex		Age (years)	BMI (g/cm²)	Disease duration (years)	Mobility		Ventilation	
	M	F				Ambulant*	Wheelchair-bound	Yes	No
Adults	17	19	51.9±11.4	24.2±3.8	17.1±9.5	25(7)	11	13	23
Children / adolescents	6	4	11.3±5.5	18.6±3.0	6.1±4.2	8(0)	2	2	8
Total	23	23	43.1±19.8	23.3±4.2	14.9±9.7	33(7)	13	15	31

* All ambulant patients. The number of patients who were ambulant, but with aids, is placed between brackets.

Table 2 | BMD *Z*-scores of adults, children/adolescents compared with healthy individuals

	Adults	Children and adolescents
Total body	-0.77±1.30[b]	-1.11±1.48[a]
Lumbar Spine	-0.06±1.47	-1.02±1.96
Femur	-0.93±1.38[b]	–

- Not determined. Level of significance is shown by a (P<0.05) and b (P<0.01).

Of all patients, 31 (67%) had a BMD *Z*-score below – 1 and 12 (26%).of them were classified as osteoporosis/low bone mass for chronological age (*T*-score <-2.5 in adults or *Z*-score <-2 in children). A BMD *Z*-score below – 1 was found at the femoral neck in 66% of the patients. Fewer (34%) had this score at the lumbar spine.

The characteristics of the 12 patients with osteoporosis/low bone mass for chronological age are given in Table 3. Four of these patients were children/adolescents; three of them had the classic infantile form of Pompe disease. In this group of twelve patients, seven (58%) were wheelchair-bound compared with 12 (23%) in the total group.

Eight of the 10 children had BMD *Z*-score below - 1).

No significant differences were found between ambulant patients and non-ambulant patients, or between ventilated and non-ventilated patients. No correlation was found between the *Z*-score of total body BMD and disease duration. There were no significant differences for age and years of symptoms between the group of twelve patient with a *T*-score <-2.5 or *Z*-score <-2 and the total group. No significant relations emerged from the multivariate analysis of total body Z-score in relation to age, ventilation dependency, and years of symptoms.

We found *Z*-scores below – 1 and even below – 2 and *T*-scores below – 2.5 in both patients with an IVS1 c-32-13T>G/null genotype and patients with other mutations. None of the patients with classic infantile or juvenile onset disease and low bone mass for chronological age had an IVS1 c-32-13T>G/null genotype.

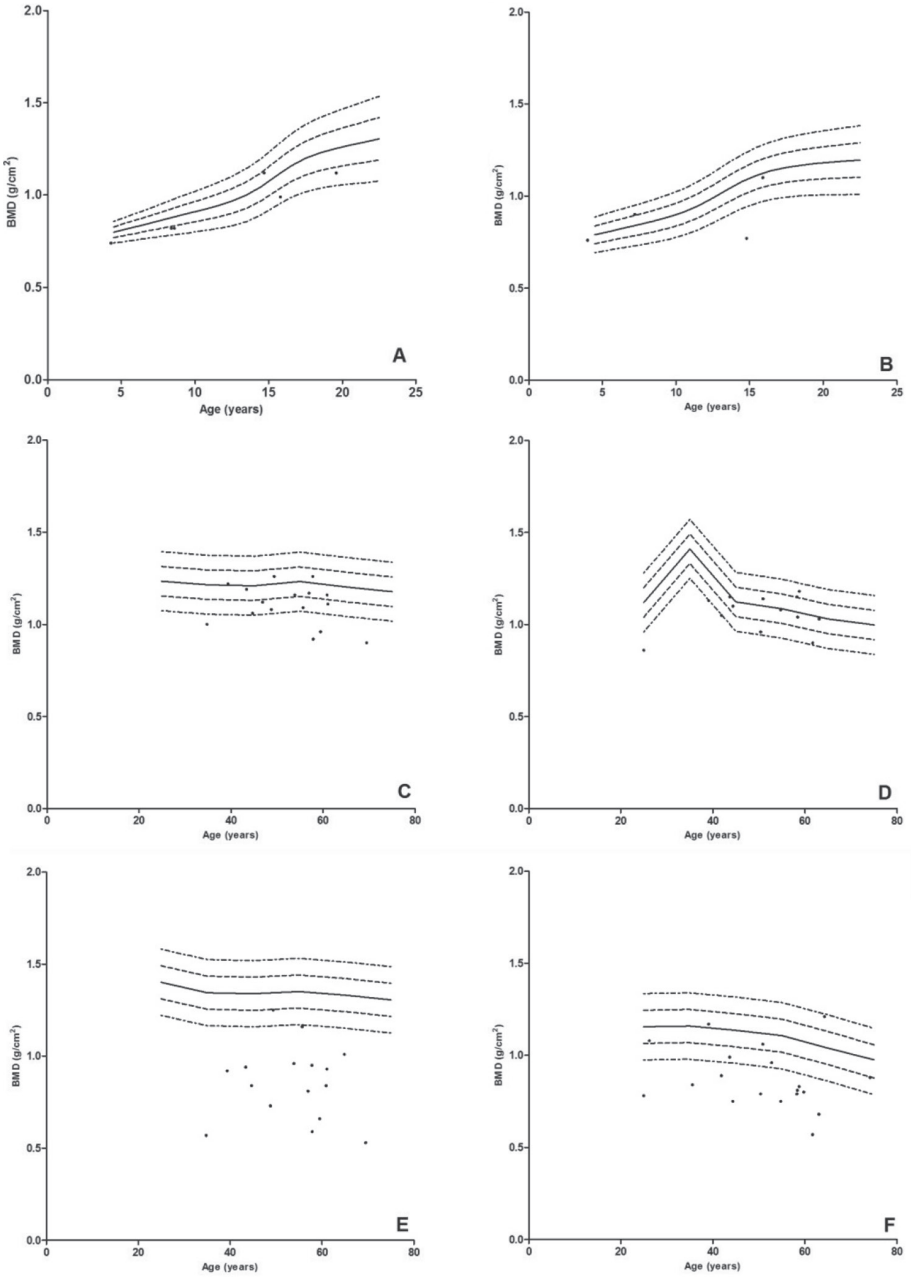

Figure 1 | Total body bone mineral density for individual patients for men (A), women (B), boys (C) and girls (D) and femoral neck bone mineral density for men (E) and women (F). Reference values are plotted for comparison. Lowest curve: *Z*=-2; next curve up: *Z*=-1; middle curve: *Z*=0; next curve up: *Z*=+1, uppermost curve: *Z*=+2.

Table 3 | Characteristics of the Pompe patients with osteoporosis/low bone mass for chronological age

Patient	Gender	Age (years)	Disease duration (years)	Classification	BMI (g/cm²)	Mobility	Ventilation	T-score			Z-score		
								TB	LS	FN	TB	LS	FN
1	M	70	40	Adult onset	24	Wheelchair-bound	Yes	**-4.0**	**-3.6**	**-4.2**	-3.2	-2.8	-2.8
2	M	60	26	Adult onset	23	Wheelchair-bound	Yes	**-3.3**	-1.5	**-3.2**	-2.9	-1.0	-2.2
3	M	58	28	Adult onset	26	Wheelchair-bound	Yes	**-3.8**	0.0	**-3.7**	-3.6	0.4	-2.8
4	M	35	11	Adult onset	20	Ambulant without aids	No	**-2.8**	-1.9	**-3.9**	-2.4	-1.7	-3.5
5	M	49	9	Adult onset	26	Ambulant without aids	No	-1.7	-0.4	**-2.7**	-2.3	-0.6	-2.4
6	F	63	27	Adult onset	25	Ambulant without aids	No	-1.2	-2.2	**-2.5**	-0.4	-1.0	-1.4
7	F	62	31	Adult onset	22	Wheelchair-bound	Yes	**-2.8**	**-2.9**	**-3.4**	-1.5	-1.4	-2.1
8	F	25	21	Juvenile onset	15	Wheelchair-bound	Yes	**-3.4**	**-3.2**	**-4.2**	-2.0	-1.8	-3.0
9	F	15	10	Juvenile onset	13	Ambulant without aids	No	–	–	–	**-4.4**	**-3.1**	–
10	M	4	4	Classic infantile	17	Ambulant without aids	No	–	–	–	**-2.0**	0.9	–
11	F	4	4	Classic infantile	16	Wheelchair-bound	Yes	–	–	–	-0.4	**-4.6**	–
12	F	7	7	Classic infantile	13	Wheelchair-bound	Yes	–	–	–	0.6	**-2.8**	–

Z-scores below -2 are bold. TB = Total Body, LS = Lumbar Spine and FN = Femoral Neck

Laboratory analysis

Analysis of the blood samples obtained from 44 patients revealed that seven patients had elevated serum concentrations of parathyroid hormone (7.6-14.4 µg/L, normal values 1.4-7.3 µg/L) and eight patients had lowered serum concentrations of 25-hydroxyvitamin D (13-47 nmol/L, normal values 50-136 nmol/L). Three of these patients had signs of secondary hyperparathyroidism, as evidenced by lowered concentrations of 25-hydroxyvitamin D in combination with an elevated serum concentration of parathyroid hormone. Only one of them had osteopenia (*T*-score <-1).

All patients had normal serum concentrations of the bone turnover markers alkaline phosphatase and β-CTx (Table 4).

No significant differences were found between the group with a *T*-score <-2.5 or *Z*-score <-2 and the other Pompe patients for the bone turnover markers and the serum concentrations of calcium, parathyroid hormone and 25-hydroxyvitamin D.

Bone mineral density and lung function

We did not find significant correlations between the BMD *Z*-scores of any region and lung function (forced vital capacity).

Bone mineral density and muscle strength

Table 5 summarizes the correlations between BMD *Z*-score of the total body and muscle strength of several regions of the body in 32 adults. Four adults were excluded from the analysis because reliable data could not be obtained for some muscle groups. A significant correlation was found between total body BMD and total body muscle strength (Figure 2). This relation remained significant after adjustment for age, ventilation dependency, and years of symptoms (P=0.025). Significant correlations were also found between the total body BMD and muscle strength of the neck region and the proximal muscles of the upper and lower extremity, no correlations were found between total body BMD and distal muscle strength.

The correlation between the strength of the proximal muscles of the lower extremities and BMD of the femoral neck showed a trend (r=0.34), but did not reach statistical significance (P=0.06). There were no significant correlations between muscle strength of any of the muscle groups and BMD of the femoral neck and lumbar spine.

Table 4 | Serum concentrations and normal values of the bone turnover markers alkaline phosphatase and β-CTx

	Patients	Normal values
Alkaline Phospatase		
Adults	68.7 (38-117)	0-119 U/L
Children <13 years	207.6 (186-231)	0-425 U/L
Boys between 13 and 17	114 (97-132)	0-455 U/L
Girls between 13 and 17	149 (85-213)	0-255 U/L
β-CTx		
Men between 30 and 50	0.13 (0.09-0.16)	0-0.57 µg/L
Men older than 50	0.18 (0.07-0.46)	0-0.70 µg/L
Women	0.24 (0.05-0.56)	0-0.56 µg/L

Table 5 | Correlation between total body bone mineral density (expressed as *Z*-scores) and muscle strength of 5 groups of muscles

	Correlation	Significance
Total body	0.47	*P*<0.01
Neck region	0.53	*P*<0.01
Proximal muscles upper extremity	0.37	*P*<0.05
Distal muscles upper extremity	-0.22	*P*=0.24
Proximal muscles lower extremity	0.43	*P*<0.05
Distal muscles lower extremity	0.33	*P*=0.07

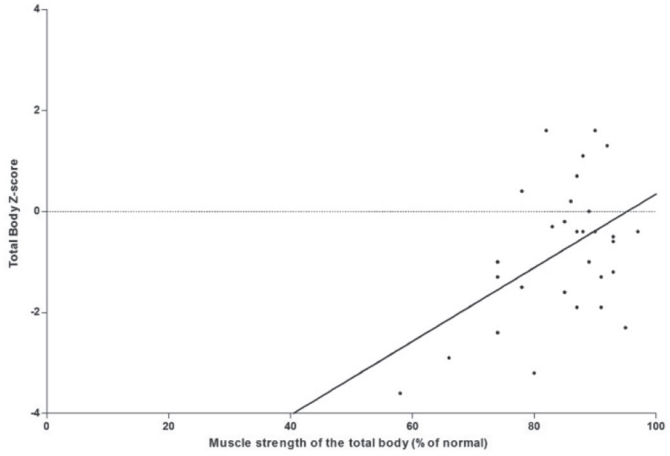

Figure 2 | Scatter plot of the *Z*-score for total body bone mineral density in adult, versus the summed score for proximal muscle strength of the total body (*n*=32). Pearson's *r*=0.43.

DISCUSSION

With the changing perspectives for Pompe disease patients, supportive care and prevention of co-morbidities such as osteoporosis are becoming more important. The results of the current study shows that .low bone mineral density, a possible indicator of the co-morbidity osteoporosis, is a common feature in patients with Pompe disease. Thirty-one of the forty-six patients (67%), that we investigated, had a BMD *Z*-score below – 1. Remarkably, this group included eight of the 10 participating children/adolescents. The eight included all four patients with the classic infantile form of Pompe disease. Three of those four had a low bone mass for chronological age (*Z*-score <-2).

Low BMD increases the risk of fractures. A recent article reported the occurrence of fractures in children with an infantile onset of Pompe disease, mostly in the long bones (femur and humerus) of patients with a lack of weight-bearing.[20] It is unlikely that patients with a decreased BMD in childhood will attain a normal peak bone mass.[24] This puts them at even greater risk for fractures later in life.

Twelve patients of the 46 patients were classified as osteoporosis/low bone mass for chronological age (*T*-score <-2.5 or *Z*-score <-2). In this group of twelve, more patients were wheelchair-bound and ventilation-dependent than in the total group of patients. This may be explained by the fact that prolonged inactivity and immobilization are important risk factors for osteoporosis.[25,26] A noteworthy finding was there was found osteoporosis/low bone mass for chronological age in five ambulant patients; this indicates that factors other than inactivity and immobilization also play a role. Muscle strength is one such factor: we found in BMD was correlated with proximal muscle strength.

The mechanostat theory postulates that muscle strength is a predictor of BMD in the non-diseased population.[27-29] Our study indicates that this theory may be extended to populations with neuromuscular diseases as well. We therefore recommend that all patients with impaired muscular strength are screened for decreased BMD. Screening should not be limited to inactive and immobile patients but should also include those who are ambulant. Skeletal muscle weakness is expected to have the greatest impact in children/adolescents, since the capacity of bone to increase its strength in response to mechanical forces is greatest during growth.[30-32]

The notion that muscle strength and weight-bearing affect BMD in patients with Pompe disease is further supported by the greater involvement of the femoral neck compared to the lumbar spine. Trabecular bone (e.g. lumbar spine) is mainly influenced by general, systemic

influences, such as hormone status and nutrition.[30] Cortical bone (the femur), however, is more subject to regional, mechanical influences, such as muscle mass and muscle strength.[30]

When treating osteoporosis/low bone mass for chronological age in Pompe disease it is important first to reduce or eliminate all the known risk factors for low bone mass. Effective control of the underlying disease is the best approach to prevent secondary osteoporosis. In 2006, enzyme therapy for Pompe disease was registered as a causative treatment for the disease. The possible long-term effects of ERT on BMD are unknown, but positive effects might be seen when muscle strength increases. Physical activity is essential to increase BMD, not only in healthy individual but also in persons with various pathological conditions.[33] On the basis of our finding that a decreased BMD is partly due to skeletal muscle weakness, we recommend exercise training for Pompe disease patients, because besides the possible positive effects on disease progression and daily life, such training may also prevent and ameliorate this long-term complication of Pompe disease. Slonim et al. advised a combination of high protein diet and exercise for Pompe disease patients.[34]

With regard to therapeutic interventions with drugs for osteoporosis/low bone mass for chronological age, one should begin with the simplest and safest ones, such as calcium and vitamin D suppletion in case of deficiency.[33] Anti-resorptive drugs such as bisphosphonates have been shown to increase BMD, relieve pain, increase mobility, and reduce fragility fractures in osteogenesis imperfecta, corticosteroid-induced osteoporosis, and osteoporosis due to cerebral palsy.[33] In children, the most commonly used are intravenous cyclical pamidronate or oral alendronate.[35-37] Although the long-term safety of bisphosphonates is uncertain, the original concerns, such as reduced healing of fractures and altered onset and course of puberty, have not been confirmed in over ten years of pediatric use.[33] Remarkably, in our patients the bone turnover markers were not increased, It is not known whether medical therapy in the form of bisphosphonates is effective in patients with Pompe disease and low BMD.

In summary, our study demonstrates for the first time that patients with Pompe disease often have a decreased BMD (*Z*-score <-1) and are at risk of osteoporosis/low bone mass for chronological age. At particular risk are patients who are wheelchair-bound and ventilator-dependent. However, in ambulant patients we also found *Z*-scores below – 1 and even below – 2 and *T*-scores below – 2.5 . We found that the low BMD of Pompe patients correlated with a decreased proximal muscle strength. For detection of a decreased BMD we recommend screening of all affected children, wheelchair-bound and ventilator-dependent adults, and all patients with decreasing muscle strength. Prospective studies are needed to evaluate the effect of ERT and exercise training on muscle strength and BMD in patients with Pompe disease.

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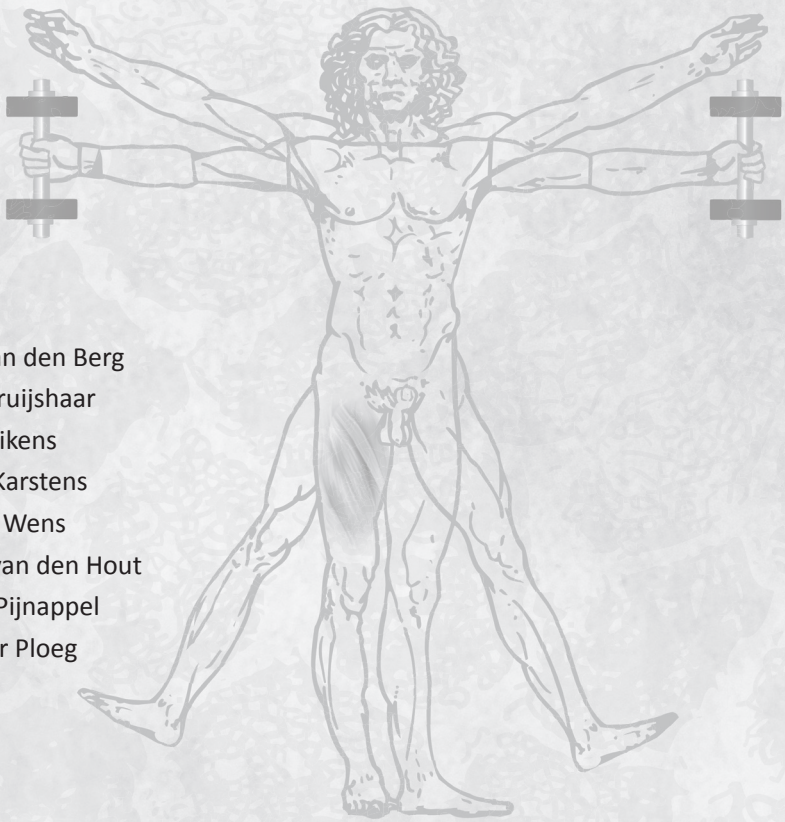
CHAPTER 5

Effects of enzyme replacement therapy on bone mass and body composition in 35 adults with Pompe disease



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ABSTRACT

Objective: Patients with Pompe disease, a rare metabolic myopathy, have a decreased bone mineral density (BMD) due to their decreased proximal muscle strength. We investigated whether enzyme replacement therapy alters patients' body composition and bone mineral status.

Methods: Body composition and BMD of adult patients were assessed at baseline and after 2-3 years of enzyme replacement therapy using DXA technology. The relationships between changes in these parameters and in muscle strength and pulmonary function were explored.

Results: 35 patients were enrolled in the study. At baseline, patient's lean mass/height^2 (LBM) was lower than of healthy individuals (*Z*-score -1.40, *P*<0.01) and slightly decreased during treatment (to *Z*-score -1.49, *P*=0.04), while fat mass/height^2 (FMI) was normal and did not change. Thirteen patients were classified as obese according to FMI compared to 3 according to BMI, their number changing little with treatment. BMD for total body and femoral neck was significantly reduced before, and did not improve during, treatment. BMD of the lumbar spine did increase during treatment but remained within the normal range (*Z*-score -0.01 to 0.14, *P*=0.01). Changes in BMD *Z*-scores were not related to change in muscle strength or pulmonary function.

Conclusion: Two to three years of enzyme therapy did not improve BMD nor body composition and did not reflect the small increase in muscle strength. Furthermore, patients with Pompe disease appeared to have a lower lean mass for their height compared to healthy individuals. It should therefore be noted that calculation of BMI underestimates fat mass.

INTRODUCTION

Pompe disease (glycogen storage disease type II, acid maltase deficiency) (OMIM # 232300) is an inherited metabolic myopathy caused by a deficiency of the lysosomal enzyme acid α-glucosidase. It leads to glycogen accumulating in a variety of tissues, most markedly in muscle cells, which in turn brings muscle cell damage and weakness.[1-4] Pompe disease presents as a broad clinical spectrum, ranging from a rapidly progressive infantile phenotype that results in death within the first year of life,[5,6] to more slowly progressive forms in children and adults with proximal muscle weakness and respiratory problems.[7-11]

Pompe disease is an orphan disease with an estimated frequency of 1 in 1:40.000 patients. Earlier we showed, that children and adults with Pompe disease have a decreased bone mineral density (BMD) and are at risk of osteoporosis.[12] Low BMD was correlated with decreased proximal muscle strength. Similar data were reported by Papadimas et al. in smaller groups of patients.[13] Osteoporosis is a known risk factor for bone fractures, which have been reported to occur as a complication of the disease in infants and children with Pompe disease. In adults it can also be expected, subsequent to decreased bone strength owing to reduced BMD, reduced weight bearing and/or muscle strength, and perhaps nutritional factors and metabolic effects of the disease.

Since 2006, enzyme replacement therapy (ERT) with recombinant human acid alpha-glucosidase is available for the treatment of patients.[14-24] Pompe diseases has thereby become the first inheritable muscle disease for which a therapy has become available. The therapy was registered on base of data in infants. In 2010 the first placebo controlled trial in adults was published. Since then ERT has shown to elicit positive effects on survival, walking distance, respiratory function, skeletal muscle strength and fatigue.[19,23,25-29] In this way ERT might influence BMD, since BMD and proximal muscle strength have shown to be correlated in Pompe disease.[12] Similarly ERT might influence body composition since an increased muscle strength might be caused by an increased muscle mass. Furthermore patients with an increased muscle mass may become more active thereby decreasing their fat mass. Earlier studies on the effect of ERT on body composition in smaller groups (*n*=9 and *n*=17) of Pompe patients were inconclusive, one study demonstrating an increase in body mass index (BMI) and fat mass (FM) after treatment (30) the other showing little effect.[31]

The goal of this study was therefore to examine the effect of ERT on patients' body composition and bone mineral status in a larger cohort of adult patients with Pompe disease.

METHODS

Patients and study design

This single-center, cohort study on the effects of ERT on body composition and bone mineral status was conducted from January 2007 to February 2011 at the Center for Lysosomal and Metabolic Diseases, Erasmus MC University Medical Center, Rotterdam. This is the national referral center for Pompe patients in the Netherlands. Patients were eligible for inclusion if:

1. diagnosis of Pompe disease was confirmed by enzyme analysis in leukocytes or fibroblasts[32] and by mutation analysis,[33]
2. they were aged 18 years or older,
3. they were able to make the transfer to the DXA table,
4. they were treated with ERT
    a. DXA scans were available at:
    b. Baseline: a maximum of 4 weeks before and 33 weeks after start ERT, AND
    c. Follow-up: between 2 and 3 years (104-156 weeks) after start of treatment .
    d. The study protocol was approved by the Medical Ethical Committee at Erasmus MC University Medical Center. Written informed consent was obtained from all patients.

Clinical assessments

Anthropometrics

Body weight was measured with indoor clothing but without shoes on a digital scale (ServoBalans type KA-20-150S, Servo Berkel Prior B.V.) to the nearest 0.1 kg. The height of ambulant patients was measured barefoot in standing position (Ulmer Stadiometer nach Prof. E. Heinze, Busse Design + Engineering); wheelchair-dependent patients were measured while lying in bed. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared. Subjects were classified according to BMI into underweight (<18.5), normal (18.5-24.9), overweight (25-29.9) and obese (≥30).

Body composition and bone densitometry

Body composition and bone mineral density (BMD) were measured conform DXA technology using a Lunar DPX densitometer and analyzed with Encore 2002 software (GE Lunar DPX, GE Health Care). Body composition was described by the mineral bone, fat, and fatty free body mass (kilograms). Derivative index values (fat mass/height^2 (FMI) and lean mass/height^2 (LBM)) were calculated and expressed as *Z*-scores. *Z*-scores are, the number of standard deviations

above or below the mean reference values for healthy persons matched for age, sex, height, weight, and ethnic group.[34] Subjects were also classified according to the fat mass index (FMI) based on the ratio of their fat mass to height squared into fat deficit (<3 for men, <5 for women), normal (3-6 for men, 5-9 for women), excess fat (>6-9 for men, >9-13 for women) and obese (>9 for men, >13 for women).[34] Bone densitometry was performed in a standardized manner as described before.[12] The BMD results were expressed as *Z*-scores, with scores lower than or equal to -1 SD for total body, lumbar spine and/or femoral neck being defined as indicating osteopenia. The *T*-score, the number of standard deviations above or below the mean for a healthy 30 year old adult of the same sex and ethnicity, was used to identify osteoporosis. A *T*-score for total body, lumbar spine and/or femoral neck *T*-score of less than or equal to -2.5 SD was defined as indicating osteoporosis.

Laboratory analysis

Non-fasting blood samples for serum concentrations of calcium (normal values: 2.20 to 2.65 mmol/L in adults), phosphate (normal values: 0.80 to 1.40 mmol/L in adults), 25-hydroxyvitamin D (normal values: 50-136 nmol/L), and parathyroid hormone (normal values: 1.4 to 7.3 pmol/L) were analyzed. In addition, the following markers of bone turnover were determined: total and bone-specific alkaline phosphatase (bone formation; normal values total 38-117 U/L, and bone-specific <20.1 µg/L in men, <14.3 U/L in premenopausal women, and 22.4 U/L in postmenopausal women) and β-CTx (bone resorption; normal values <0.57 µg/L in men between 30 and 50 years, <0.70 µg/L in men older than 50 years, and <0.56 µg/L in women). β-CTx concentrations were determined using an electrochemiluminescence immunoassay (ECLIA) following the manufacturer's instructions (ß-CrossLaps/serum, Cobas®, Roche Diagnostics, Mannheim, Germany).

Lung function

Forced vital capacity was measured using spirometry in upright position and expressed as percentage of predicted normal values as described previously.[10]

Muscle strength

Muscle strength was assessed using Hand-Held Dynamometry (HHD) in a standardized manner and sum scores calculated as described before (total muscle score encompassing neck flexors, neck extensors, shoulder abductors, elbow flexors, elbow extensors, wrist extensors, hip flexors, hip abductors, knee flexors, knee extensors, foot dorsiflexors, and foot plantiflexors;

proximal muscle score for the upper extremities: shoulder abductors, elbow flexors, and extensors; proximal muscle score for the lower extremities: hip flexors and abductors, knee flexors, and extensors).[12]

Statistical analysis

Data were expressed as means with standard deviations (SD). To assess whether patients´ *Z*-scores for body composition and BMD were significantly different from healthy individuals (*Z*-score 0) at baseline and during ERT the one-sample *t*-test was used.

Comparison of mean differences in body composition and BMD parameters before and during ERT was made using the paired-sample *t*-test for normally distributed data. For not normally distributed data, the Wilcoxon signed rank test for paired sampled was used. Analyses were stratified by subgroups described in previous studies:[10,14,23] gender, age (<45 years and ≥45 years old), disease duration (<15 years and ≥15 years), wheelchair use, ventilation use and FVC in upright position (≥80% and <80%), as well as by treatment duration (≤13 weeks and >13 weeks) and baseline BMD *Z*-score (≤-1 and >-1). Pearson correlation coefficients were calculated in order to ascertain the relationships between the clinical parameters (muscle strength and lung function) and BMD.

The number of patients with osteopenia and osteoporosis and the distribution of patients across the different BMI and FMI classification groups before and after the intervention were compared using the chi-square test. All analyses were performed using SPSS for Windows (version 17.0, SPSS Inc. Chicago, IL, USA). Two-sided P-values less than 0.05 were considered significant.

RESULTS

Patients

A total of 35 patients (16 males, 19 females) fulfilled the inclusion criteria and participated in this study. Their mean age at the start of ERT was 50.6 years, 26% used a wheelchair, and 29% used mechanical ventilation (Table 1). The median follow-up period was 119 weeks (range 106-151). In our previous study on bone mineral density, 46 patients with Pompe disease were included.[12] For the present study, only adult patients were selected. This resulted in the exclusion of the 15 younger patients who participated in our previous study. Furthermore, four new adult patients, diagnosed after completion of the previous study (but with sufficient follow-up), were included.

Table 1 | Baseline patient characteristics

Sex		Age (years)	Disease duration (years)	Treatment duration (years)	Mobility		Ventilation	
male	female				ambulant[a]	wheelchair-bound	yes	no
16	**19**	51±12	17±10	12±8	26 (6)	**9**	**10**	**25**

[a] All ambulant patients. In parentheses: the number of patients who were ambulant, but with aids.

Body composition

Body composition could be analyzed for 33 adult patients; weight at follow-up was missing for two patients. At baseline, patients had a significantly lower mean lean body mass/height^2 (LBM) compared to healthy individuals (mean *Z*-score -1.40, *P*<0.01), while their mean fat mass/height^2 was within the normal range of healthy individuals (Table 2). Classification of patients according to their body mass index (BMI) and FMI gave different results: three patients were classified as obese according to BMI in contrast to 13 patients according to FMI.

After a median duration of 2.3 years of ERT, lean mass/height^2 had decreased slightly (*Z*-score from -1.40±0.73 to -1.49±0.69, *P*=0.04) , while patients' weight and fat mass/height^2 did not change (Table 2). Compared to baseline the number of obese patients according to BMI seemed to decrease, whiles this was not reflected by FMI.

Bone mineral density

Table 3 shows the BMD results. Patients' baseline bone density values for total body and femoral neck remained within the range considered normal for healthy individuals (*Z*-score between -1 and 1). Despite this, the negative *Z*-scores were significantly different from 0 indicating, as described in our previous study,[12] that Pompe patients have significantly lower bone density than the healthy population.

Table 2 | Comparison of body composition before and after treatment with ERT for two years

	Before ERT	After ERT	*P*-value*
Height (cm)	174.8±10.5	174.8±10.4	0.33
Weight (kg)	74.8±12.6	75.0±14.0	0.78
Lean body mass/height^2 (*Z*-score)	-1.40±0.73†	-1.49±0.69†	0.04
Fat mass/ height^2 (*Z*-score)	0.13±0.90	0.17±0.93	0.46
Classification according to BMI (*n*)			
Underweight (<18.5)	1	1	
Normal (18.5-24.9)	19	19	
Overweight (25.0-29.9)	10	12	
Obese (≥30)	3	1	
Classification according to FMI (*n*)			
Fat deficit (M<3, F<5)	1	1	
Normal (M 3-6, F 5-9)	5	6	
Excess fat (M>6-9, F>9-13)	14	12	
Obese (M>9, F>13)	13	14	

* For the difference before and after ERT. † Significant difference between *Z*-score with healthy individuals (*P*<0.01). *n*: number

Table 3 | Comparison of bone mineral density measured by a DXA scan before and after treatment with ERT for two years

	Baseline (-4 - +33 from start ERT)	Follow-up	*P*-value*
Total Body			
Bone mineral density (*Z*-score)	-0.69±1.11†	-0.73±1.19†	0.42
Lumbar Spine			
Bone mineral density (*Z*-score)	-0.01±1.44	0.14±1.44	0.01
Femoral Neck			
Bone mineral density (*Z*-score)	-0.79±1.29†	-0.93±1.21†	0.72
Classification of patients (*n*)			
Osteopenia: *Z*-score ≤-1	19	17	0.65
Osteoporose: *T*-score ≤-1	5	5	1.00

* For the difference before and after training. † Significant difference between *Z*-score with healthy individuals (*P*<0.01). *n*: number

During follow-up, the BMD of the total body and femoral neck did not change. A significant increase was observed in bone mineral density of the lumbar spine (*P*=0.01). The *Z*-score, however, remained within the normal range and did not differ significantly from the healthy population scores at baseline nor at follow-up (*P*=0.51and *P*=0.56, respectively). Subgroup analysis showed that the increase in lumbar spine BMD was significant in men but not in women (*Z*-score from -0.16 at baseline to +0.08 during ERT, *P*=0.01 versus +0.14 to +0.20, *P*=0.37; difference between the subgroups *P*=0.05). The number of patients who met the criteria for osteopenia reduced by two. One patient was no longer osteoporotic, while another patient became osteoporotic.

Bone mineral density in relation to clinical parameters

Calcium concentrations were within the normal range at start of ERT and remained so after two years of ERT. At baseline one patient had reduced concentrations of 25-hydroxy vitamin D in combination with an elevated serum concentration of parathyroid hormone. These signs of secondary hyperparathyroidism persisted after two years of ERT. This patient had a normal BMD at both measurements. All patients' serum concentrations of the bone turnover markers alkaline phosphatise and β-CTx were normal at start of ERT and remained normal during ERT.

FVC in upright position was below normal reference values at baseline and remained the same at follow up (*P*=0.35). Sum scores for muscle strength of the total body, improved slightly from 89.4% of normal compared to healthy individuals to 91.1% of normal (*P*=0.02).

No significant correlations between the change in the BMD Z-scores and the change in FVC or in muscle strength were found.

DISCUSSION

This cohort study of 35 adult patients with Pompe disease shows that two to three years of treatment with ERT did not improve body composition or bone mineral density. Body composition showed a small decrease in the already low lean body mass per height squared. Bone density remained low for the total body and femur, while values for the lumbar spine did increase but remained within the normal range. Since the contribution of mineral bone mass to lean body mass is little we conclude that the relatively low lean body mass is most likely due to a decreased muscle mass. As a result, classification according to BMI understimates obesity in this patient population. Indeed accroding to BMI 9% of patients was obese prior to starting ERT, while this was 39% according to FMI.

This finding thus supports the thesis by Ravaglia et al. that BMI assessment may underestimate the increase in fat mass in Pompe disease patients due to muscle substitution by intramuscular fat.[25,30]

Given the relationship between muscle strength and BMD and the observed increase in muscle strength with treatment we would have expected to find a positive effect of ERT on BMD.[12,35,36] It is possible that we were not able to observe such an effect yet, since too little time has passed since muscle strength has started to increase. For example, as reported bij Sievanen et al. follow-up after injury of an otherwise healthy, physically active 26-year old woman not affected with Pompe disease, showed that BMD recovered more than one year after muscle strength was fully recovered.[37] In patients with Pompe disease we observed that increase of muscle strength commenced within 3 to 9 months after start of ERT. Recovery of muscle strength is however limited and full recovery is not likely to occur in many patients.[14] In addition to this, DXA technology may not be able to detect early changes of bone remodeling. The mechanical competence of bone is a function not only of its intrinsic material properties (mass, density and stiffness) but also its structural properties (size, shape and geometry). The two-dimensional skeletal outcome achieved by DXA visualizes only the material properties of overall bone strength, whereas changes in the structural properties have shown to occur earlier.[38,39] The use of high resolution peripheral quantitative computed tomography can detect such structural properties and might help to identify these processed more accurate in future. Finally, bone remodeling starts first and is more evidently in trabecular bone than in peripheral bone.[39,40] In this study we showed a significant increase of lumbar spine BMD which is trabelcular bone/It might be envisaged that an increase of BMD of the femoral neck, which is peripheral bone, comes later.

In addition to the above, a gain in muscle strength alone may not be enough to increase BMD. In order to optimally stimulate bone remodeling, patients should also actively use their muscles so that pulling forces are applied to the bones. While a certain level of physical activity may not be sufficient to maintain bone health, these might not suffice to improve bone density. Resistance training exercises seem to facilitate these improvements,[41] while at the same time exerting a positive effect on patients well-being.

Despite the increase in muscle strength observed in patients during treatment with ERT, their lean mass continued to decrease slightly. In theory it might be expected that increased strentgh should have led to an increased muscle volume and as a result increased bone density. One of the explanations might be that it has been reported that ERT has the largest effects in those patients who are least affected and who have little or no muscle atrophy and that it shows its most prominent effect in the least affected fibers.[3,42,43]

In conclusion, patients with Pompe disease have a lower lean mass for their height compared to healthy individuals. Therefore, calculation of BMI underestimates their fat mass and we suggest that FMI should be used preferably in clinical practice if one wants to get insight in true body composition. No clinically meaningful improvement in bone density nor body composition was observed after 2-3 years of enzyme therapy, despite an observed small increase in muscle strength. Longer follow-up is needed.

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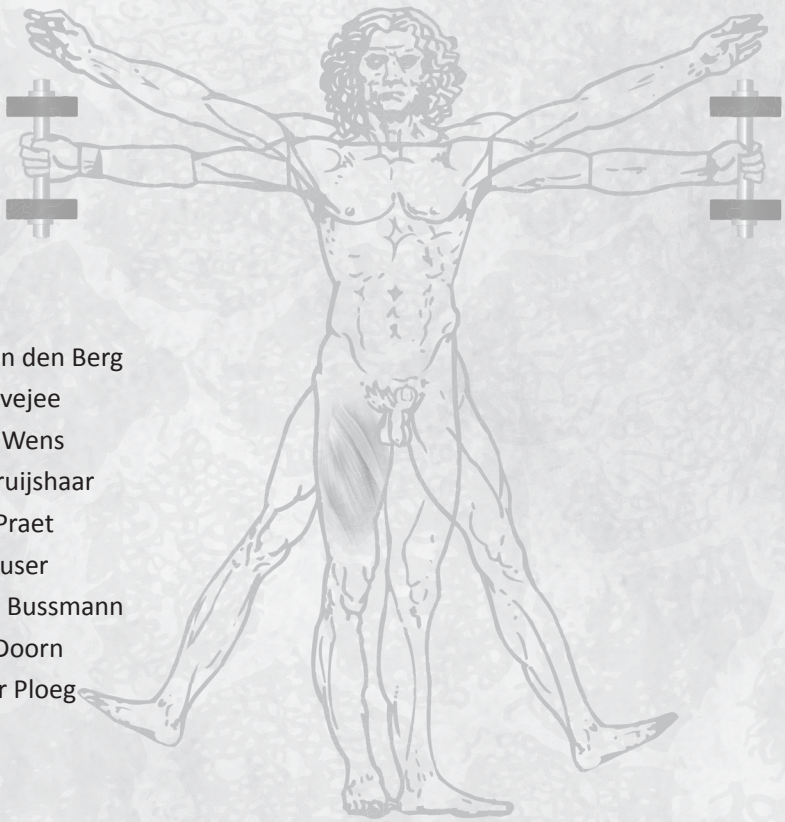
CHAPTER 6

Safety and efficacy of exercise training in 23 adult with Pompe disease receiving enzyme therapy



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ABSTRACT

Objective: Pompe disease is a proximal myopathy. We investigated whether exercise training is a safe and useful adjuvant therapy for mildly affected adults receiving enzyme replacement therapy (ERT) for this disease.

Methods: Training comprised 36 sessions of standardized aerobic, resistance and core stability exercises over 12 weeks. Before and after, we evaluated safety, endurance, muscle strength, muscle function, core stability, and body composition.

Results: Of 25 patients enrolled, 23 successfully completed the training. Improvements in endurance were shown by increases in maximum workload capacity (100W±52 to 122W±53, $P<0.01$), maximal oxygen uptake capacity (69.4% of normal±17.4 to 75.9%±18.0, $P<0.01$), and maximum walking distance (6 minute walk test: 492 meters±89 to 508±97, $P=0.01$). There were slight increases in total muscle strength (hand-held dynamometry), mainly due to an eight percentage point increase in hip flexors *(*$P<0.01$). Functional tests showed small reductions in the time needed to climb four steps (0.3 sec, $P=0.02$) and rise to standing position (1 sec, $P=0.05$), while time to run and the quick motor function test results remained unchanged. The number of patients who were able to perform the core stability exercises rose, as did the core stability balancing time ($P<0.05$, for all four exercises).

Conclusions: Our study shows that a combination of aerobic, strength and core stability exercises is feasible, safe and beneficial to mildly affected adults with Pompe disease. It should therefore be considered as an adjuvant treatment in Pompe patients receiving long-term ERT.

INTRODUCTION

Pompe disease (glycogen storage disease type II, acid maltase deficiency) (OMIM # 232300) is a metabolic myopathy caused by glycogen accumulation resulting from deficiency of lysosomal acid α-glucosidase (*GAA*). It presents as a wide clinical spectrum, the most prominent symptoms in adults being proximal skeletal muscle weakness and respiratory problems.[1,2] Skeletal muscle weakness typically fits a pattern of limb-girdle myopathy, with the abdominal and paraspinal muscles and the musculature of the hip being the most affected muscle groups.[3-5]

Enzyme replacement therapy (ERT) with recombinant human acid α-glucosidase (Myozyme/Lumizyme) was approved for the treatment of Pompe disease in 2006. In adults, ERT has been shown to elicit positive effects on skeletal muscle strength, walking distance, respiratory function and survival.[6-16]

Patients' fitness and physical functioning may be further supported by treatments additional to ERT, such as exercise training. Although some recent studies suggest that exercise training may be beneficial, evidence is still limited.[12,17]

A recent study on common clinical practice in the Netherlands showed that there is a lack of uniformity in the type of physical therapy training programs practiced applied, and that physical therapists and patients all seek guidance and standardization.[18] We therefore aimed to determine whether a standardized and well-structured exercise intervention program combining aerobic, resistance and core stability exercises was feasible and safe, and whether it added value to treatment with ERT alone. In a group of relatively mildly affected adult Pompe patients who had been receiving ERT for more than a year, we evaluated the effects of such a regime on endurance, muscle strength and function, core stability, and body composition.

METHODS

Patients

Patients were recruited at the Centre for Lysosomal and Metabolic Diseases, Erasmus MC University Medical Centre, Rotterdam, the Dutch national referral centre for patients with Pompe disease.

There were three inclusion criteria:

1. A confirmed diagnosis of Pompe disease measured by decreased acid α-glucosidase activity in leukocytes or fibroblasts, and also by mutation analysis;
2. Age >17 years;
3. Treatment with ERT for at least 52 weeks

There were four exclusion criteria:

1. The use of walking-aids or a wheelchair;
2. Ventilator-dependency;
3. Concurrent medical conditions;
4. Participation in other exercise-training programs.
5. The study was approved by the Ethical Committee at Erasmus MC University Medical Centre. Informed consent was obtained from all patients.

Study design and intervention

Three times a week for 12 weeks, all patients followed a standardized training program that was provided under the supervision of physical therapists at carefully selected sports or fitness centres near the patients' homes. To ensure the uniformity of the training program and its supervision, all therapists attended a one-day instruction program at Erasmus MC University Medical Centre. The training program is depicted in Figure 1. The first training session was on-site supervised by one of the researchers from Erasmus MC (LvdB, MF), who subsequently attended each training site every two weeks to monitor the proper conduct of the program.

Patients were randomly subdivided into two groups: group 1 (n=13), which started the program at week 1; and group 2 (n=12), which started at week 13. The staggered start of training allowed us to investigate whether any improvement observed in the training period could also be attributed to ERT.

WARM-UP

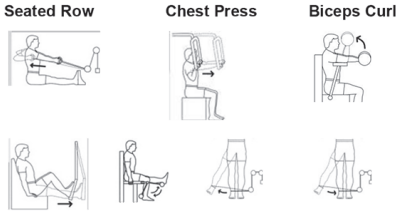
Duration:	5 minutes
Intensity:	HR 100-110 BPM

ENDURANCE TRAINING

Duration:	15 minutes
Intensity:	HR corresponding 60% $VO_{2\,MAX}$

STRENGTH TRAINING

Initial weight:	70% of 4 RM
Nr. sets:	3
Nr. repeats:	15-20
Adjustment of weight:	↓ if 1 set <15 repeats ↑ if 3 sets >20 repeats

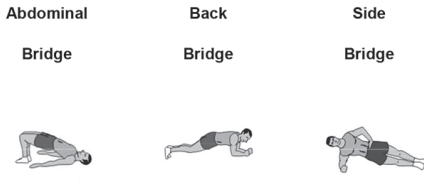


ENDURANCE TRAINING

Duration:	15 minutes
Intensity:	HR corresponding 60% $VO_{2\,MAX}$

CORE STABILITY TRAINING

Nr. sets:	3
Duration per set:	30 sec
Adjustment of intensity:	↓ if 1 set <30 sec ↑ if 3 sets >30 sec



COOLING-DOWN

Duration:	5 minutes
Intensity:	HR 100-110 BPM

To assess the effects of the program, each patient visited our centre (Erasmus MC) on two separate days in weeks 0, 12 and 24. The co-primary endpoints of this study were endurance, muscle strength and muscle function. Secondary endpoints were core stability and body composition.

Under the supervision of the physical therapist, training diaries were kept by all patients, who recorded the days on which they trained, the weight and number of repeats of resistance exercises, and the perceived level of exertion. To evaluate training progress and patients' motivation, patients were telephoned weekly.

Assessments

Safety

Plasma CK was measured every two weeks as a safety marker for exercise-induced muscle damage,[19,20] and patients were contacted every week to record potential side effects such as pain and fatigue.

Endurance

Aerobic exercise capacity was determined using an incremental cycle ergometer test. After four minutes of unloaded cycling on the cycloergometer (Jaeger ER 800; Erich Jaeger, Würzburg Germany) exercise intensity was increased progressively until exhaustion (i.e. ramp protocol), during continuous measurement of patients' heart rates and ventilator parameters using spiroergometry equipment (Oxycon Pro, Jaeger, Würzburg, Germany). At exhaustion, the rating of exertional symptoms was assessed using the Borg scale (scale 6-20).[21] Maximum workload capacity (W_{MAX}) and, peak oxygen uptake capacity ($VO_{2\ PEAK}$) were measured. The ventilatory threshold (VT) was assessed independently by two clinical exercise physiologists using the ventilatory equivalents method.[22,23] The test was considered to be maximal when one of the following criteria was met:[23]

1. heart rate was >90% of that predicted,
2. respiratory exchange ratio (RER) was >1.11, or
3. VO_2 stabilized despite increased workload.

Forced vital capacity was measured using spirometry in upright position, and was expressed as a percentage of predictive normal value as described previously.[14] Finally, walking distance on the 6-minute walk test (6MWT) was evaluated according to the American Thoracic Society guidelines.

Muscle strength

Muscle strength was assessed by one investigator (SW) using Manual Muscle Testing (Medical Research Council (MRC) score,[24] and Hand-held Dynamometry (HHD). Assessments were performed in a standardized manner, and sum scores were calculated as described previously.[14]

Muscle Function

Functional activity assessments comprised three timed tests: 10 meter running, climbing four steps, and rising from supine to standing positions,[25] and also the Quick Motor Function Test (QMFT), a test specifically designed and validated for Pompe patients.[26] The QMFT consists of 16 specific motor skills related to daily activities. A total score is achieved by summing the scores for each item (ranging from 0 "cannot perform" to 4 "can perform with no effort"), and is expressed as a percentage of the maximum score.

Core Stability

To assess the dynamic balance, which reflects core stability, one physical therapist (MF) measured time in balance (in seconds) for each of the four core stability exercises of the training program (Figure 1).[27,28]

Body composition

Bone mineral density (BMD) and body-composition measurements were performed conform DXA technology using a Lunar DPX densitometer and analyzed with Encore 2002 software (GE Lunar DPX, GE Health Care). Bone densitometry was performed in a standardized manner as described previously.[29] Body composition was described in terms of the mineral, lean and fat body mass (kilograms). The percentage of fat mass and, more specifically, android and gynoid fat, were calculated.

Statistical analysis

Patient characteristics were summarized using descriptive statistics. Data for the two groups were combined after verifying that there were no significant differences between outcome measures before the start of the training (group 1 – week 0; group 2 – week 12; student's *t*-test was used for normally distributed data, and Mann-Whitney for not-normally distributed data).

Mean differences before and after the training were compared using the paired *t*-test for normally distributed data, and otherwise the Wilcoxon signed rank test for paired samples.

For group 2, we also used these tests to compare the outcome measures before and after the control period (week 0 to 12).

Significance level was set at $P<0.05$. Statistical analyses were performed using SPSS for Windows (release 17.0; SPSS, Inc., Chicago, IL).

RESULTS

Patients

A total of 25 patients fulfilled the inclusion criteria and chose to participate in this study. Two patients did not complete the training program because it was too time-consuming for them. This left 23 patients, who successfully completed the study.

Their ages ranged from 20 to 71 years (median of 46 years). They had been receiving ERT for 1 to 6 years with a median of 3 years (Table 1). Patients in the two randomly assigned groups were comparable in terms of age, gender, disease duration, time on ERT, and number of training sessions completed. As the results of the assessments did not differ significantly between the two groups at start of training ($P\geq0.1$), we could analyze the effects of the training study in the total group of 23 patients.

Safety

During the first week of training, two patients had a high plasma CK level (10125 U/l and 6149 U/l), and also experienced muscle pain and fatigue. Over the following week, their CK-values dropped to their normal range, and the fatigue and pain disappeared. Both patients continued training. None of the other patients had pain, fatigue, or increases in plasma CK levels during the study period.

Table 1 | Patient characteristics

	Group 1 (*n*=12)	**Group 2 (*n*=11)**	**Total group (*n*=23)**	***P*-value***
Male gender (%)	7 (58%)	5 (45%)	12 (52%)	0.54
Age in years (range)	45.4 (19.6-70.5)	46.6 (32.9-66.1)	46.0 (19.6-70.5)	0.85
Disease duration in years (range)	15.5 (8.1-28.1)	16.1 (6.0-32.1)	15.8 (6.0-32.1)	0.83
ERT duration in years (range)	3.3 (1.4-6.5)	3.0 (1.3-3.6)	3.1 (1.3-6.5)	0.96
Training sessions (max. 36)	33 (27-36)	32 (24-35)	32 (24-36)	0.70

Group 1 trained in weeks 1-12 and Group 2 trained in weeks 13-24. For the difference between group 1 and 2 (chi-2 test for proportions and Wilcoxon signed rank test for continuous data); ERT = enzyme replacement therapy.

Endurance

All patients were able to complete the incremental cycle test without adverse events. One was excluded from the analysis because he did not reach the required maximum intensity defined in the method section. After 12 weeks of training W_{MAX}, VO_{2peak} and VT/kg improved significantly (Table 2). There were no significant differences between patients' maximum heart rates before and after 12 weeks of training, indicating that the results were truly based on an increase in fitness rather than on greater exertions by the patients towards the end of the training period. FVC did not change. Average walking distance on the 6MWT increased by 16 meters (*95% CI* 4.4-27.7, *P*=0.01).

Muscle strength

There were slight increases in the strength of the proximal lower extremity muscles and in total strength measured by HHD (both *P*=0.01, Table 3). These increases were due mainly to an eight percentage point increase in the strength of hip flexors (*P*<0.01). Assessment by MMT produced no significant changes in strength.

Muscle function

Twelve weeks of training significantly reduced the time taken by patients to climb four steps (on average 0.3 seconds less, range -2.16 - +0.19, *P*=0.02, Table 4) and to rise from supine to a standing position (1 second less, range -9.09 - +1.65, *P*=0.05). The QMFT sum score and the time to run 10 meters did not change.

Table 2 | Aerobic fitness measured in an incremental cycle test and a 6-min walk test before and after 12 weeks of training

	Before training Mean ± SD	After training Mean ± SD	*P*-value**
Incremental cycle test (*n*=22*)			
Maximum workload (W_{max}, Watt)	110±52	122±53	<0.01
Maximum heart rate (bpm)	156±25	161±20	0.16
Peak oxygen uptake (VO_{2peak}, % of normal)	69.4±17.4	75.9±18.0	<0.01
Forced vital capacity (FVC, % of normal)	89.2±12.6	90.0±14.0	0.51
Ventilatory threshold (VT/kg, ml/min/kg)	16.7± 4.3	18.5±4.7	<0.01
6-min walk test (*n*=22)			
Maximum walking distance (6MWT, m)	492±89	508±97	0.01

* One patient was excluded because he did not reach the required maximum intensity. ** For the difference before and after training (paired samples *t*-test).

Table 3 | Muscle strength measured by hand-held dynamometry (HHD) and manual muscle testing (MMT) before and after 12 weeks of training

	Before training Mean ± SD	After training Mean ± SD	*P*-value*
HHD sum score of (*n*=23)			
all muscles [xx] (% of normal)	87.3±7.3	88.6±7.6	0.01
proximal muscles of the upper extremity[‡] (% of normal)	96.7±4.5	97.3±3.5	0.27
proximal muscles of the lower extremity[†] (% of normal)	77.2±13.5	79.3±14.0	0.01
MRC sum score (% of normal) (*n*=22**)	89.5±6.7	89.9±5.7	0.52

* For the difference before and after training (paired samples t-test and the Wilcoxon signed-ranks test for paired data). xx: ‡, † and neck extensors and neck flexors. ‡: Proximal muscles of the upper extremity used for the sum score: shoulder abductors, elbow flexors and elbow extensors. † Proximal muscles of the lower extremity used for the sum score: hip flexors and hip abductors, knee flexors and knee extensors. ** MRC sum score was not available for one patient

Table 4 | Muscle function measured by the quantitative motor function test (QMFT) and timed tests before and after 12 weeks of training

	Before training Mean ± SD	After training Mean ± SD	*P*-value*
QMFT score (*n*=22**)	51±8	51±9	0.65
Timed Tests (*n*=22**)			
10 m running (sec)	4.97±1.50	4.70±1.34	0.16
Climbing four steps (sec)[‡]	2.37±0.80	2.08±0.74	0.02
Rising from supine to standing position (sec)[‡]	5.83±4.25	4.83±2.38	0.05

*For the difference before and after training (paired samples *t*-test and the Wilcoxon. signed-ranks test for paired data[‡]).
** QMFT score and timed test were not available for one patient.

Core stability

Figure 2 shows the results of the core stability tests. At the start of the training program, many patients experienced difficulties in performing the core stability exercises, reporting problems with initiating movement and with controlling balance in the right position. During the training program, the number of patients who were able to perform the exercises increased for three of the four exercises (from 17 to 21 for the abdominal bridge, and from 15 to 16 for the left side bridge, and 13 to 16 for the right side bridge). The average time they were able to remain in balance improved for all four positions. Time in balance increased by 58% for the back bridge , by 229% for the left and 223% for the right side bridges, and by 86% for the abdominal bridge; $P<0.05$).

Body composition

There were no changes in mineral bone mass (2.83 kg ± 0.58 before training vs 2.82 kg ± 0.57 after training), in lean body mass (42.53 kg ± 7.99 vs 43.14 kg ± 8.28), or in fat mass (30.11 kg ± 9.23 vs 29.29 kg ± 9.06). Likewise, there were no changes in bone mineral density, overall fat percentage, and android and gynoid fat percentages (results not shown).

ERT only

For the 12 weeks before their training started, patients in group 2 (ERT only) underwent the same set of assessments as in the 12 weeks of training (ERT plus training). This enabled us to use group 2 to compare the effects of ERT only with the combined effects of ERT and training. During the first 12 weeks of ERT only, we detected no significant improvements in the main outcome measures.

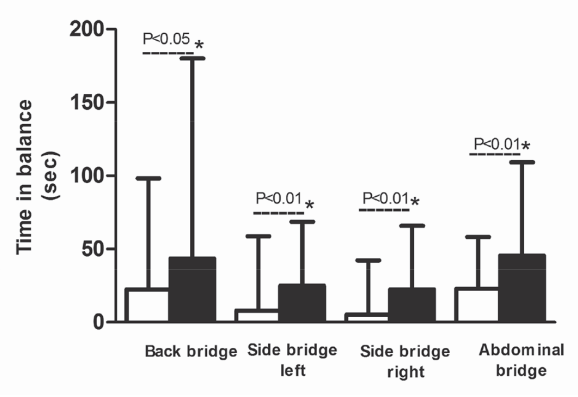


Figure 2 | Time patients (*n*=23) were able to remain in balance for the four different core stability exercises before (white bars) and after training (black bars).

DISCUSSION

This is the first study providing clinical evidence that a combination of aerobic, resistance and core stability training is feasible and can not only be performed safely in Pompe patients who are treated with ERT, but also helps to improve endurance, muscle function and core stability, and, to some extent, muscle strength. Such a program thus has added value for mildly affected Pompe patients receiving long-term enzyme replacement therapy.

Aerobic fitness improved over 12 weeks of training. In addition to the 10% increase in maximum workload capacity, peak oxygen uptake and ventilatory threshold improved by 7% and 11%. So far the 6MWT was used in clinical trials for Pompe disease to assess endurance capacity, but since most patients have walking difficulties it has been questioned whether the 6MWT fully reflects endurance capacity.[30,31] Our study indicates that the incremental cycle test offers a good alternative to test endurance capacity, providing insight into patients' aerobic capacity.

The increase we measured in the total HHD muscle strength sum score was small (one percentage point), and was caused mainly by the improvement in strength of the hip flexors, which showed the largest effect. We are not completely certain whether these improvements in strength of the hip flexors resulted from strength training, the core stability exercises, or both. Core stability training is aimed at teaching individuals to use their muscles adequately, and may thereby support gains in muscle strength once adequate proprioception and coordination is accomplished.

Core stability has not been trained previously in neuromuscular disorders presenting with limb-girdle weakness. One possible reason may lie in the assumption that core stability exercises are not feasible for patients with such disorders. Indeed, when the training program began, many patients had difficulty performing the core stability exercises. During the program, however, they learned to activate the proper muscle groups and were able to remain in balance for longer. Our results thus indicate that core stability training is feasible and improves time in balance in patients with Pompe disease who are treated with ERT. Feedback from patients during the training also suggests that they perceived their improved core stability to facilitate daily activities. Further studies on how training influences patient reported outcomes such as quality of life are needed.

The increases in endurance, muscle strength and core stability also led to some functional improvement, with patients becoming able to climb four stairs and rise to a standing position faster.

Compliance was high in our study, due largely to the supervision by physiotherapists, the weekly telephone consultations, and the beneficial effects the patients experienced. While dropout rates might be greater outside the setting of this study, all patients except two continued training two times a week after it ended, reflecting the positive feedback they had given us on the program. To avoid drop-out in general practice, we recommend that the program be incorporated into regular supervised physiotherapy sessions.

Few studies have been conducted on exercise and training in Pompe disease. The largest so far was performed before ERT became available.[17] For a mean duration of four years, the

26 patients included in it participated in a combined nutrition and endurance exercise therapy program that led to improved muscle function as measured with the Walton score (0.29 points lower annual increase). More recently, an observational study on the effects of ERT on walking distance showed that this effect was the most pronounced in five of the 44 patients who had been subjected to endurance training on a cycle ergometer during ERT infusions.[12] Two other studies investigated the effects of endurance exercise during ERT infusion;[32,33] one was performed in mice and the other in five adult patients; neither could confirm the beneficial effects observed earlier.

Most of the exercise studies in Pompe disease focused on endurance training, since it was envisaged that resistance training might lead to muscle damage, thereby aggravating muscle weakness.[34-36] An exception is a study by Terzis et al.,[12] in which 5 patients followed a 20-week aerobic-resistance training program combined with ERT. At group level, muscular strength and walking distance both improved.

Aerobic training studies amongst small groups of patients with neuromuscular dystrophies and McArdle disease, a metabolic myopathy like Pompe disease, have been published.[37-40] The increases in VO_{2peak} (ranging from 12-47%), were slightly higher in these studies than in ours, and might be explained by the greater total time patients spent on endurance training per week. A second explanation might be the lower baseline VO_{2peak} than in our study. Both aspects may have affected the magnitude of the training response on VO_{2peak}.

Before starting our study we carefully considered whether we should perform exercise-endurance training only, or a combination of different types of exercises. We chose the latter, because we wanted not only to improve endurance, but also to target all affected muscles (resistance exercises), and to ameliorate proprioception and the strength of proximal muscles not targeted by resistance training (core stability exercises). Our decision to use a combined program was also driven by the fact that our patient population was not large enough to run three separate programs.

Although we cannot rule out the possibility that an exercise program of aerobic fitness training alone might have had a greater impact on endurance, we observed that the extra exercises had positive effects on core stability, and may also have improved muscle strength and muscle function. Earlier studies of training in patients with inherited muscular myopathies did not include core stability exercises; our study shows them to be both safe and easy to learn for mildly affected patients with Pompe disease. These exercises might be beneficial also for other neuromuscular diseases.

CONCLUSION

Our study shows that a combination of aerobic, strength and core stability training is feasible and can be performed safely in mildly affected patients with Pompe disease. Such training helps to improve endurance, muscle strength, muscle function and core stability. This training program thus seems to offer added value for Pompe patients receiving long-term ERT.

Patients in our study were mildly affected. Those who are more severely affected may need adjusted exercise-training programs. Since our patients benefited particularly from a combination of endurance and core-balance exercises, and may also have benefited from resistance training, we recommend that these are incorporated into comprehensive exercise training programs for these patients. It should be noted that training should always be performed under the strict supervision of medically trained physical therapists.

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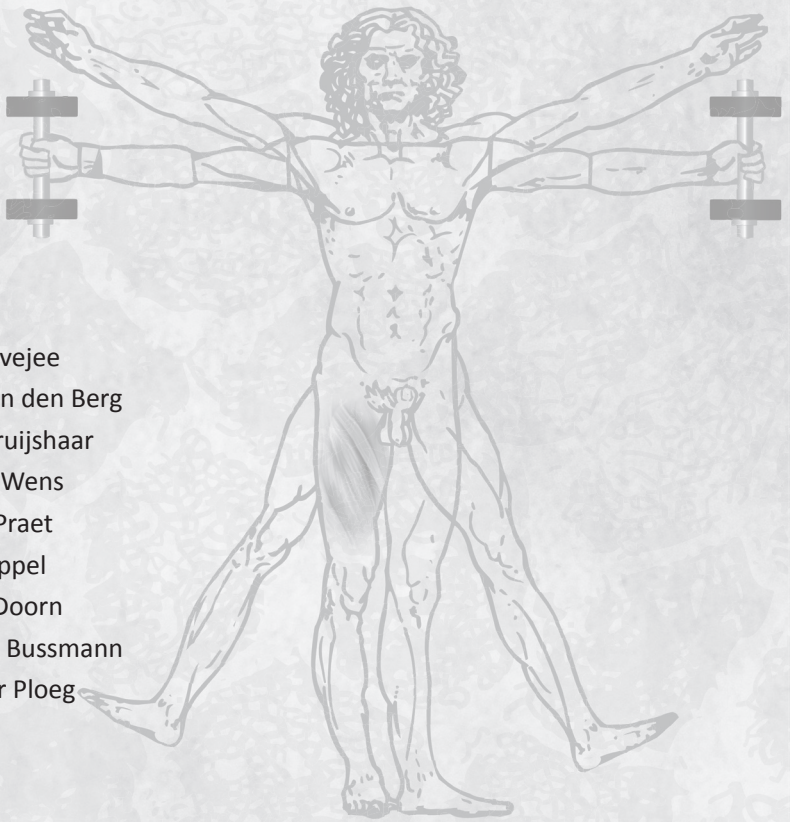
CHAPTER 7

Exercise training in adults with Pompe disease: the effects on pain, fatigue, and functioning



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ABSTRACT

Pompe disease is an inherited neuromuscular disorder characterized by a progressive skeletal and respiratory muscle dysfunction. Other important symptoms include pain and fatigue. We previously showed a combined training program aimed at increasing aerobic fitness, muscle strength and core stability to improve these parameters. The goal of this study was to assess if this program also affects fatigue, pain, activity and participation, and if so, assess the relationship of these effects with trained parameters.

Patients undertook a 12 week exercise programme, which included 36 sessions of standardized aerobic, resistance and core stability exercises. Before and after the training program, we evaluated fatigue (FSS), pain (yes/no), motor function (QMFT, R-PAct), volume of physical activity (ActiGraph) and quality of life (SF-36; PCS, MCS). Relations between changes in trained parameters and in additional outcome measures were studied using correlation coefficients.

Of the 25 patients enrolled, 23 completed the program. Significant changes in fatigue (5.33 to 4.78, *P*=0.007) and pain (56.5% to 21.7%, *P*=0.040) were shown. Additionally a borderline effect on mental health was found (56.1 to 59.1, *P*=0.055). The quality of motor function and volume of physical activity did not change. Significant relations were found between both improvement in aerobic fitness and muscle strength, and mental health, while these were not related to pain and fatigue.

This paper shows that a combined training program aiming to increase aerobic fitness, muscle strength and core stability also leads to improvements in fatigue and pain, and tends to improve mental health in mildly affected Pompe patients.

INTRODUCTION

Pompe disease is an inheritable metabolic disease caused by a deficiency of the lysosomal enzyme acid alpha-glucosidase, which results in a glycogen accumulation in cells. The disease has a broad clinical spectrum; in adult patients it primarily affects skeletal and respiratory muscles.[1]

Since 2006 enzyme replacement therapy (ERT) with recombinant human acid α-glucosidase has been available. While ERT has been shown to improve skeletal muscle strength, walking distance, respiratory function and survival in adult patients[2-5] complementary therapies, such as exercise therapy may further enhance patients' functioning and quality of life. A few studies have demonstrated a positive effect of exercise therapy in Pompe disease.[4,6-8] However, insufficient conclusions can be drawn by the combination of exercise training with other adjuvant therapies and the small number of patients included in most studies. Our recently reported controlled training study further strengthens the evidence for a beneficial effect of exercise training.

In this study, 23 mildly affected Pompe patients receiving ERT completed a training program consisting of a combination of aerobic, resistance and core stability exercises (Chapter 6). This training regime resulted in significant improvements in outcomes directly related to the intervention, such as aerobic fitness, muscle strength and core stability as well as in distance walked. These outcome measures mostly represent functioning at the body function and structure level as described in the International Classification of Functioning, Disability and Health (ICF).[9] They do, however, not cover the activity and participation level of this classification, i.e. the functioning of a person as a whole and functioning in a social context, which are also key to the assessment of functioning. Pompe disease strongly affects patients' ability to carry out daily life activities and participation and is furthermore associated with fatigue and pain, suggesting that these are indeed important parameters to take into account in the evaluation of treatment for this disease.[10-12]

The aim of this paper is to present the effect of the combined training programme on pain, fatigue, activity and participation. In addition, we studied correlations between all domains of the ICF model, in order to gain insight into the extent to which functional measures were related to the direct training goals.

METHODS

Patients

Patients were recruited at the Center for Lysosomal and Metabolic Diseases, Erasmus MC University Medical Center, Rotterdam, which is the national referral centre for patients with Pompe disease in the Netherlands. The study was approved by the Ethical Committee of the Erasmus MC University Medical Center; all patients provided informed consent. Inclusion criteria were a confirmed diagnosis of Pompe disease, age above 17 years, receiving ERT for at least 1 year, and not dependent on ventilator and/or walking devices. Concurrent medical conditions and participation in other exercise-training programs were exclusion criteria.

Intervention

The standardized training program is described in detail by vd Berg et al. (Chapter 6). All patients were trained under supervision of physiotherapists at carefully selected outpatient clinics. Briefly, the programme consisted of 3 supervised training sessions per week, during a 12-week period, composed of a combination of aerobic, resistance and core stability exercises.

Measurements and outcome measures

To assess the effects of the program, each patient visited our centre, Erasmus MC, on two separate days at baseline and after 12 and 24 weeks. Besides fatigue and pain patients' functioning was assessed at the activity and participation level. Patients were asked to fill in questionnaires evaluating fatigue, pain, and functioning at activity and participation level. In addition, an Actigraph device was used to assess patients' volume of physical activity.

Fatigue

The severity of fatigue and its impact on an individual's daily functioning was assessed using the Fatigue Severity Scale (FSS). 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 are indicative of fatigue and scores of 5 and higher of severe fatigue.[10,13] We used the validated Dutch translation of the FSS.[13]

Pain

Two items were dedicated to assess the presence of pain/ cramps (are you suffering from pain or cramps; yes/no) and its severity (how severe is your pain or cramp; very mild, mild, moderate, severe, very severe).

Activity level

Functioning at activity level was further subdivided into motor function and physical activity volume. Within the activity level, 4 tests were included:

- the 6 minute walk test (6 MWT),[14] as described bij vdBerg (Chapter 6)
- the total score of the quick motor function test (QMFT),[15] as reported by vd Berg (Chapter 6).
- Pompe-specific Activity Scale (R-PAct scale).[16] This scale covers 18 activities of daily living, which are reported to be the most important and limiting activities for Pompe patients. Activities range from combing hair to running, using the Rasch-built self-reported Pompe-specific Activity Scale (R-PAct scale).[16] The R-PAct score is calculated as the mean item score and translated to a scale from 0 to 100, with higher values representing better functioning, as described.[16]
- all patients were fitted with an ActiGraph GT3X (ActiGraph, Pensacola, FL) accelerometer to provide objective measures of the volume of physical activity. Patients were instructed to wear the ActiGraph, mounted on an elastic belt around the waist with the unit positioned over one of the hips during waking hours. The ActiGraph monitors were set to activity counts in tri-axial mode, using a 10-second epoch, which were reintegrated to 60 seconds for analyses. Non-wear time was assessed using a minimum of 60 minutes of consecutive zero counts allowing up to a 2 minute tolerance of non-zero counts. A minimum of 8 hours per day of wear time on at least three days was required to be included in analysis.[17]

Quality of life

Quality of life was assessed using The SF-36 health survey v.2.[18] This generic health status questionnaire contains 36 items that measure eight dimensions (bodily, pain, physical function, social function, role limitations because of physical problems, role limitations because of emotional problems, mental health, vitality; general health perceptions). Norm-based scores were calculated for each of the domains and the two summary scores: the physical component summary (PCS) and mental component summary (MCS) score. These scores vary between 0 and 100, with higher values representing better function.[19] A score of 50 represents the general population mean.

Aerobic fitness, muscle strength and core stability

Three types of test were done at the level of body structure.

- Aerobic fitness was determined by measuring (VO_{2peak}) and maximal workload (W_{max}) through an incremental cycling test to exhaustion.[20]
- Muscle strength was measured by hand-held dynamometry (HHD) as described previously.[2]
- In addition, core stability was evaluated before and after training, by measuring time in balance (in seconds) for each of the four core stability exercises of the training program.[21,22]

Statistical analysis

Median differences in the outcomes parameters before and after the intervention were compared using the Wilcoxon signed rank test for paired samples. For the comparison of dichotomous outcome we used the Chi-square test. In order to assess the extent to which functional measures were related to the direct training goals (i.e. aerobic fitness, muscle strength and core stability) we investigated the correlations between changes (before versus after training) in the outcome measures of different ICF domains using the Spearman correlation coefficient. Significance level was set at $P<0.05$. Statistical analyses were performed using SPSS for Windows (release 20.0; SPSS, Inc., Chicago, IL).

RESULTS

Patient characteristics

Twenty-three of the 25 patients enrolled in this study completed the training programme. Their median age was 46 year, and they had been treated with ERT for a median of 3 years (Table 1). Table 2 shows the previously reported outcome measures at body structure level (Chapter 6).

Effects of 12 weeks of training

Fatigue decreased significantly from a median FSS score of 5.33 before training to 4.78 after twelve weeks of training ($P=0.007$; Table 3). Pre-training, 77% of the patients were fatigued (FSS score>4) and 59% were severely fatigued (FSS score>5). After completing the training programme this was 74% and 44%, respectively.

Table 1 | Patient characteristics

	Group 1 (*n*=12)	Group 2 (*n*=11)	Total group (*n*=23)	*P*-value*
Male gender (%)	7 (58%)	5 (45%)	12 (52%)	0.54
Age in years (range)	45.4 (19.6;70.5)	46.6 (32.9;66.1)	46.0 (19.6;70.5)	0.85
Disease duration in years (range)	15.5 (8.1;28.1)	16.1 (6.0;32.1)	15.8 (6.0;32.1)	0.83
ERT duration in years (range)	3.3 (1.4;6.5)	3.0 (1.3;3.6)	3.1 (1.3;6.5)	0.96
Training sessions (max. 36)	33 (27;36)	32 (24;35)	32 (24;36)	0.70

* For the difference between group 1 and 2 (chi-2 test for proportions and Wilcoxon signed rank test for continuous data); ERT = enzyme replacement therapy

Table 2 | Previously published outcome measures at body structure level (mean values and SD) before and after 12 weeks of training (Chapter 6)

	Before training	After training	*P*-value
Aerobic fitness (maximum oxygen uptake (VOmax; % of normal); *n*=22**	69.4±17.4	75.9±18.0	<0.01*
Muscle strength (HHD sum score of all muscles; % of normal)	91.5±4.9	92.4±5.0	0.01*
Core stability (average of all items ‡; sec)	15.4 (0.0-96.2)	28.9 (0.0-88.3)	<0.01#
Maximum walking distance (6MWT; m); *n*=22**	492±89	508±97	0.01*
Muscle function (total score QMFT); *n*=22**	50.8±8.1	51.2±8.5	0.65*

HHD = hand held dynamometry; QMFT = quantitative motor function test; 6 MWT: six minute walk test. * For the difference before and after training (paired samples *t*-test). **#** For the difference before and after training (paired samples *t*-test and the Wilcoxon signed-ranks test for paired data. ‡ Core stability: sum score of all items = (back bridge+ prone bridge+ left side bridge+ right side bridge)/4. ** VO_{max}, 6 MWT and QMFT score were not available for one patient

The number of patients reporting pain also decreased significantly after 12 weeks of training (13/23 vs 5/23; *P*=0.007). Before training 7 patients reported moderate pain and 6 mild pain, while after training 5 patients reported mild pain and none moderate pain. No patients reported severe pain at either time point.

No significant differences were observed in self-reported motor function, measured by the R-PAct. The volume of physical activity, measured as the mean number of counts per day using the Actigraph device did not change after 12 weeks of training at group level (see Table 3). Just over half of our patients (9/16) showed a decrease in their physical activity; the remaining 7 patients showed an increase.

Median scores and ranges for the physical (PCS) and mental (MCS) component summary scores are also shown in Table 3. A borderline improvement was seen in the MCS (56.1 vs 59.1; *P*=0.055).

Table 3 | Comparison of fatigue, pain, activity and participation level (median values and ranges) before and after 12 weeks of training

	Before training	After training	*P*-value*
Fatigue (FSS score)	5.33 (2.11;6.56)	4.78 (1.78;6.67)	0.007
Pain (number, %)	13/23 (56.5%)	5/23 (21.7%)	0.040
Motor function (R-Pact score)	70.0 (54.0;100.0)	70.0 (48.0;89.0)	0.490
Volume of physical activity (Actigraph) (x 10^3) in counts *n*=17	484,7 (163,4; 709,8)	418,3 (151,2; 905,3)	0.212
Qol (SF-36 PCS score)	39.84 (24.4;53.2)	42.14 (21.1;50.6)	0.855
Qol (SF-36 MCS score)	56.1 (25.4;68.6)	59.13 (34.4;69.0)	0.055

QoL = quality of Life. * For the difference between before and after training (chi^2 test for proportions and Wilcoxon signed rank test for continuous data).

Correlations between different outcome measures

Table 4 displays the correlations between changes in the outcome measures that were trained (aerobic fitness, muscle strength and core stability) on the one hand, and fatigue, pain, motor function, physical activity volume, and participation on the other. Improvements in pain and fatigue were not correlated to changes in any of the trained outcome measures.

With regard to motor function, the improved walking distance on the 6 MWT was significantly correlated with improvements in aerobic fitness and muscle strength. The QMFT correlated borderline with improvement in aerobic fitness (*P*=0.06). No significant correlations between the volume of physical activity as measured by the AG and aerobic fitness, or muscle strength were seen.

Table 4 | Correlations between changes in different outcome measures before and after training

Body level			**Activity level**				**Participation level**	
	Fatigue	**Pain**	**Motor function**			**Physical activity volume**	**QoL (SF-36)**	
	FSS		**6 MWT**	**QMFT**	**R-Pact**	**AG**	**PCS**	**MCS**
Aerobic fitness (VO_{2max})	-.149	.325	.495*	*.414*#	-.120	-.120	-.173	.657**
Muscle strength (HHD)	-.021	.286	.448*	.310	.005	.005	-.282	.517*
Core stability	.060	-.161	.245	-.072	.209	.271	-.042	.331

VO_{2max} = Maximum oxygen uptake HHD = hand held dynamometry; FSS = fatigue severity score; 6 MWT = 6 minute walk test; QMFT = quick motor function test; R-Pact = Rasch-built Pompe-specific Activity scale; AG = Actigraph; QoL = Quality of Life; PCS = physical component summary; MCS = mental component summary. **Significant *P*<0.0; * significant at *P*<0.05; # borderline

At participation level significant correlations (0.01 up to 0.001) were found between the MCS of the SF-36 and improvement of aerobic fitness and muscle strength. No significant correlations were found with the physical component score of the SF-36.

No correlations were observed between changes in any of the outcomes at the activity or participation level and improvements in core stability.

DISCUSSION

We previously showed that a combined training program of aerobic, resistance and core stability exercises improved parameters directly related to the intervention (i.e. aerobic fitness, muscle strength and core stability) as well as the distance walked, in a group of mildly affected adult Pompe patients (Chapter 6). The present paper adds that this training program also significantly improved fatigue and pain, and tended to improve mental health. The combined training programme did not affect the quality of motor function nor the volume of physical activity. Changes in mental health were associated to changes in aerobic fitness and muscle strength, while this was not the case for fatigue and pain.

Fatigue is an important feature of non-classic Pompe disease and has been reported as one of the disease's most disabling symptoms.[10] Before the start of our study, 77% of participants indicated to be fatigued, and 59% to be severely fatigued. Our training program significantly decreased the level of fatigue by 0.5 points (FSS from 5.33 to 4.78; *P*=0.007). Similar to the study of Güngör et al.[11] we found no correlations between reductions in fatigue and improvements in muscle strength, nor in the other trained parameters. Therefore, this finding does not support our assumed chain of subsequent effects, with exercise leading to improved exercise capacity, which in turn lowers physical load in daily life, resulting in less fatigue. Training may thus affect fatigue through another route. For example, it has been suggested that exercise influences the neuro-endocrine system and levels of neurotransmitters positively,[23] which may reduce the perception of fatigue. Further research is needed to elucidate the underlying mechanisms of fatigue and training in Pompe patients.

Pain is another complaint frequently reported by Pompe patients and impacts on their well-being and quality of life.[12,24,25] Our training program positively affected pain, reducing the proportion of patients experiencing pain from 57% at baseline to 22% after training. Pain in Pompe disease may have various causes, including short-term peak loading of weakened or atrophied muscles, and more long-term mechanical stress. This stress may result from postural

problems: muscle weakness and imbalance of strength in the contralateral muscles increases the amount of biomechanical stress that patients place on their musculoskeletal system.[26] However, we could not explain the reduction in pain by improvements in muscle strength in our patient group. Whether this is because of the relatively small number of patients, the modest increase in muscle strength (1% point in total muscle strength, or if another mechanism plays a role remains to be determined. Nevertheless, irrespective of the explanation, the pain reduction itself is important for Pompe patients.

While the distance walked as described by vd Berg (Chapter 6) improved after training, the program did not improve the other motor function parameters, neither self-reported (R-PAct and PCS) nor clinically observed (QMFT). In other words, training seems to have a positive effect on the speed of movement (distance walked in 6 minutes), but not on the quality of motor function (QMFT and R-PAct), i.e. how activities are performed. The increased aerobic fitness may allow patients to move more quickly, while still having difficulties in executing these movements without using compensatory strategies. It is also possible that the period of 12 weeks is too short to achieve changes in the way patients carry out specific movements.

Except for the 6 MWT, we found no correlations between motor function outcomes and the parameters directly related to the intervention. A possible explanation may be that the exercises did not target specific motor tasks, such as walking explicitly. In post-stroke patients task-specific training complementary to exercise therapy has been shown to have added value improving the performance of activities of daily life.[27,28] Outcomes may be optimized by adding task-specific exercises, like gait training, in which the focus is on coordination and execution. The observed correlation between the improved walking distance on the 6 MWT with both endurance and muscle strength can be explained by the fact that the 6 MWT is a combined assessment of cardiac, respiratory, circulatory and muscular capacity.

So far, little information was available about the volume of physical activity of Pompe patients, i.e. how active these patients are in daily life. It seems likely, given the impairments of the disease, that patients are less active. Indeed, prior to training, we found that the level of physical activity, expressed in mean number of counts per day, was considerably lower (203.4 10^2) than in healthy controls (269.7 10^2), and comparable to the level observed in mildly affected multiple sclerosis patients (204.7 10^2).[29] Similar to the other activity outcomes, this outcome did not improve with training. In addition to the reasons mentioned above, this outcome reflects habitual behaviour, that may be difficult to change and might needs additional strategies, such as coaching, motivational interviewing, and a more tailored approach in general. On the other hand, we found that the volume of activity did increase

in a subset of patients. Before starting training, these patients were less fatigued, had better core stability, and higher QMFT and R-Pact scores. Although the sample size is too small to perform sub-analyses, this may suggest that this training program does affect the volume of physical activity in patients with a certain minimum level of capacity. Although this should be interpreted with caution, less frequent training and/or a program with lighter exercises might be more suitable for more severely affected patients.

Generally, exercise is known to improve QoL.[30,31] The possible improvements in mental health observed in this study are in agreement with studies in other diseases that claim that exercise training is associated with an improvement in this domain of QoL.[31,32] However, it should be noted that the MCS at baseline is similar to the standard scores. The correlations between these improvements and the effect of the training on both muscle strength and aerobic fitness are in line with findings in healthy subjects[32] and chronically diseased populations.[28,30] However, psychological factors, such as attention of researchers and physiotherapists, may also play a role.

Our study is the first to focus on the effects of training in Pompe patients on a wider range of parameters including activity and participation. Limitations of our study are the relatively short duration of training and small number of patients. Longer training and follow-up is needed to confirm if continued exercise is required to maintain the effects of training and to see if patients can continue training independently outside of the program. A point of concern is that our study did not have a randomized controlled design. It is therefore possible that other aspects of our intervention (e.g. frequent attention) contributed to the observed improvements, and results should be interpreted with caution.

CONCLUSION

This paper shows that our combined training program aiming to increase aerobic fitness, muscle strength and core stability also leads to improvements in fatigue and pain, and tends to improve mental health in mildly affected Pompe patients. The program did not increase the quality of motor function or the volume of activity. The observed improvements in pain and fatigue were not determined by aerobic fitness, muscle strength or core stability. Further research is needed to understand the functional consequences of Pompe and the underlying mechanisms of exercise.

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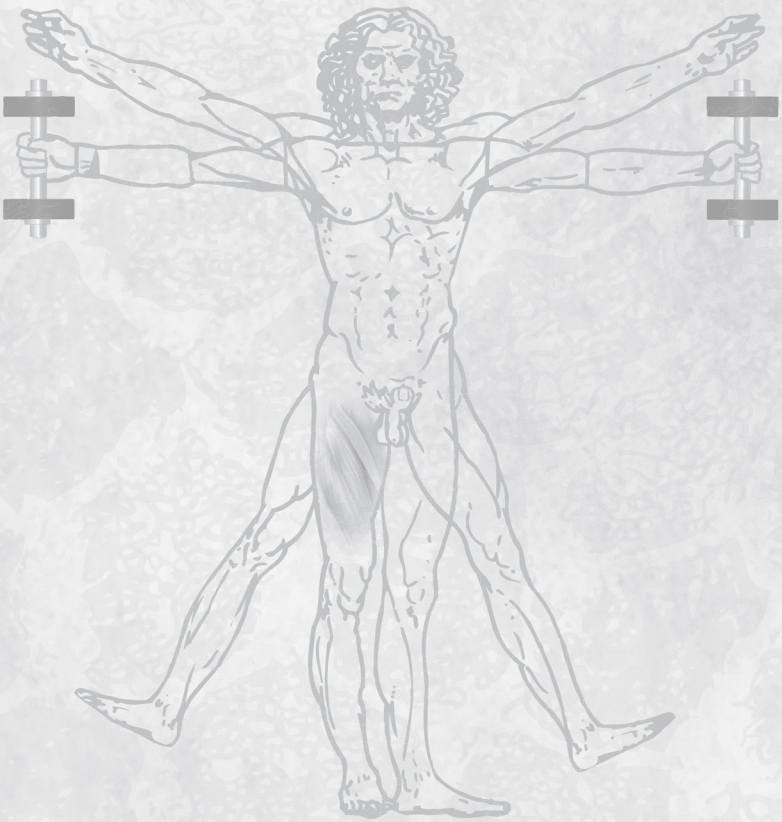
CHAPTER 8

General Discussion



Pompe disease is the first myopathy for which, with the introduction of enzyme replacement therapy (ERT) in 2006, treatment became available. Although the availability of ERT has changed patient's perspectives in terms of improved survival, increased muscle strength and stabilisation of pulmonary function, not all patients respond equally well, and not all muscle damage and functional impairment is resolved. This thesis explores i) to what extent muscle fiber-type distribution and fiber-type-specific damage contribute to the clinical diversity in Pompe disease; ii)) whether patients with Pompe disease have decreased bone mineral density (BMD) and what the effect of ERT is on BMD and body composition; and iii) whether the condition of patients with Pompe disease can be safely improved by a standardized exercise program combining aerobic, resistance and core stability exercises, in addition to ERT.

This chapter summarizes and discusses the main findings and their clinical implications along with future perspectives.

Main findings

- Overall, patients with Pompe disease have the same muscle fiber-type distribution as healthy persons, but the more severely affected patients have a greater proportion of type 2x muscle fibers than the less affected patients;
- Patients with Pompe disease have a decreased BMD and are at risk for osteoporosis;
- The low BMD correlated with decreased proximal muscle strength and did not improve during two to three years of ERT;
- Patients with Pompe disease also have a low lean body mass, which is most likely due to a low muscle mass and was not affected by 2-3 years of ERT either, despite some increase in muscle strength;
- The use of a combined training program, consisting of aerobic, strength, and core stability training is feasible and safe in mildly affected patients with Pompe disease;
- The training program helps to improve endurance, muscle strength, muscle function and core stability, and also leads to improvement in fatigue, pain and possibly mental health.

Fiber-type distribution and fiber-type-specific damage

Pompe disease presents as a broad clinical spectrum.[1,2] The clinical phenotype depends to a large extent on the level of residual acid α-glucosidase activity resulting from the pathologic genotype. Epigenetic and modifying environmental factors must exist, but have not yet been identified.

In search for these factors, the muscle biopsy of a patient who presented in our clinic with an uncommon combination of symptoms was studied. The patient appeared having selective involvement of type 1 muscle fibers (**Chapter 2**). This led us to hypothesize that differential involvement of muscle fiber types might contribute to the clinical heterogeneity in Pompe disease and potentially also cause variable response to ERT.

In this context, we studied the muscle fiber-type distribution and the fiber-type-specific damage in a larger group of patients before they received ERT (**Chapter 3)**. This showed that the muscle fiber-type distribution of the patients was overall the same as of healthy persons. However, patients in an advanced stage of disease, either having classic infantile Pompe disease or other phenotypes, seemed to have a greater proportion of type 2x muscle fibers than patients with less advanced disease. This new finding could be typical for Pompe disease, but could equally reflect a more general mechanism as also observed in other diseases leading to significant reduction of physical activity.[3]

With regard to the finding of increased numbers of type 2x fibers in severely affected patients. Studies in mice and humans have shown that skeletal muscle fibers are heterogeneous in their response to ERT with type 2x fibers being the least responsive to treatment.[4-6] Together these findings suggest that more severely affected patients will respond less well to ERT than less affected patients. As a matter of fact, this is already an accepted notion, based on clinical observations.[7-10] Muscle fiber-type distribution may thus be involved in the clinical heterogeneity of patients. A mechanism has been proposed for explaining the lower responsiveness to ERT of type 2 muscle fibers compared to type 1 muscle fibers. The rhGAA that is administered as ERT enters the muscle fibers by endocytosis. The endocytic and autophagic pathways converge, and the type 2 fibers accumulate more indigestible autophagic debris than the type 1 fibers. Thus, in type 2 muscle fibers, the autophagic system as well as the endocytic system are affected blocking the efficient delivery of rhGAA.[5]

In the same study (**Chapter 3**), we failed to demonstrate quantitative differences between the muscle biopsies of severely affected and less affected patients by measuring the percentage of damaged muscle fibers and the degree of muscle fiber vacuolation with immunohistochemistry. Others could not quantify the differences either.[11-13] Despite the lack

of quantifiable differences some muscle biopsy specimens showed clearly more affected than others by visual inspection, and the biopsies with the highest percentage of damaged muscle fibers were derived from patients with the most severe proximal muscle weakness and low vital capacity.

All in all, considering the intensity of the labour required for quantitative assessment of muscle fiber type and muscle fiber-type-specific damage and the poor correlation between these parameters and the clinical phenotype, we do not recommend the application of these sophisticated technologies for the routine follow-up of patients with Pompe disease nor for investigating the effects of ERT. On the other hand more recently an automatic imaging analysis software program called MARS (muscle assessment and rating scores) was developed.[14] The software is executed automatically and was tested highly sensitive even to subtle defects of muscle architecture.[14] In the future this software program might be used to grade muscle morphology, compare biopsies and evaluate evolution on muscle diseases and its treatments.

Bone mineral density and body composition

Decreased BMD and increased incidence of fractures have been observed in several myopathies[15-17] and lysosomal storage diseases.[18,19] Also in infants and children with Pompe disease there is evidence to suggest that bone fractures occur more frequently.[20]

The results presented in **Chapter 4** demonstrate that patients with Pompe disease have a decreased BMD and are consequently at an increased risk for osteoporosis, which in turn predisposes for bone fractures. At particular risk are those patients who are wheelchair-bound and ventilator-dependent, but our data demonstrate that also ambulant patients with Pompe disease can have a very low BMD signifying osteoporosis. In addition, we found the low BMD to go hand-in-hand with reduced proximal muscle strength. The mechanostat theory postulates that in healthy individuals, bones adapt their strength to the strain posed upon them by physiological loads. Since the largest physiological loads are caused by muscle contractions, there is a close relationship between bone strength and muscle force. Our results suggest that this theory applies in Pompe disease the same as in the healthy status, and that the low BMD of Pompe patients is caused by reduced proximal muscle strength.

Consequently, with ERT eliciting a positive effect on skeletal muscle strength[9,21-23] it might also improve the BMD. However, after three years of treatment we did not observe a clinically meaningful improvement in the overall BMD (**Chapter 5**). The BMD of only the lumbar spine increased, but was not abnormally low prior to ERT. Bone of the lumbar spine differs somewhat from other bones. It is mainly trabecular bone, in which bone remodelling reacts faster to

changes and is more pronounced than in peripheral bone.[24,25] By lack of other explanations, this difference might possibly explain why, in our study, bone of the lumbar spine responds better than the other bones to ERT by increase of BMD. Notably, the 2-3 years follow-up time in our study may have been too short to register clear changes in the BMD.

Apart from the effects of ERT on BMD, this study (**Chapter 5**) also assessed its effects on body composition (i.e. fat and lean body mass). Earlier studies on the effect of ERT on body composition were done in relatively small groups of patients and provided inconclusive results. Studying a relatively large group of patients with Pompe disease (*n*=35) we found that they had a lower lean body mass than healthy individuals. This might be explained by the loss of muscle mass, which is likely to be substituted by fat mass.[22,26] As a result, the body mass index (BMI) seems not an appropriate measure for obesity in Pompe disease, and the fat mass index (FMI) might be better used. In our study and that of Ravaglia et al.,[26] the FMI was able to identify a larger number of obese patients compared to the BMI. In healthy persons, high levels of intramuscular fat can be associated with increased risk of cardiovascular disease.[27] At present, there is no reason to associate Pompe disease and patients with other muscular diseases with increased risk of cardiovascular disease, but a high FMI may predispose for it alike in people not having Pompe disease.[27] The application of ERT did not improve the patients' BMI, in fact their lean mass decreased further during treatment.

Exercise training in Pompe disease

Patients' fitness and physical functioning may be further supported by treatments that they receive in addition to ERT. Before starting a study on the safety and efficacy of exercise training in 25 adults with Pompe disease receiving ERT (**Chapter 6**) we carefully considered which exercises to include in the training program. Because we not only wanted to improve endurance, but also target all affected muscles and ameliorate proprioception we chose a combination of exercise-endurance training, resistance exercises and core stability training. This decision was further driven by the fact that our patient population was not large enough to run three separate programs.

Evaluation of this exercise training program after a 12 week period showed that the combination of aerobic, strength and core stability training, 3 times a week, is feasible and can be performed safely in mildly affected patients with Pompe disease. As intended, the training improved endurance, muscle strength and core stability (**Chapter 6**). The program also reduced fatigue and pain, and tended to improve mental health (**Chapter 7**). With respect to patients' functioning, the program increased the speed with which certain tasks were

performed (distance walked in 6 minutes, time needed to climb four steps and time needed to rise from supine to a standing position), but did not ameliorate the quality of movements neither self-reported nor clinically observed and also did not improve the patients' ability to function in their daily life or the amount of physical activity of the patients during the day. The improvement in walking distance was related to improvements in aerobic fitness and muscle strength. This increased fitness may allow patients to move more quickly, while still having difficulties in executing these movements.

FUTURE PERSPECTIVES

The treatment and care for patients with lysosomal storage disorders and myopathies remains a great challenge despite the availability of therapy and other modes of intervention in some of them. Major problems are the lack of complete understanding of the pathophysiology and the inability to restore or at best prevent damage and functional loss of critical organs like muscle bones brain and kidney. Under these circumstances, first line care remains of utmost importance for optimizing the condition of the patients while undergoing new sophisticated treatments. In Pompe disease this message translates to understanding the cause of muscle cell damage and the loss of muscle function and how muscle function can be improved by training apart from ERT. As a result of my thesis work, a number of lessons were learned that may serve as future guideline.

Muscle pathology

Knowledge about muscle pathology in Pompe disease has gradually developed since the first description of the disease in 1932; a development greatly supported by the discovery of lysosomes, the discovery of the acid α-glucosidase deficiency and the application of electron microscopy. In the past decade, after the introduction of ERT, studies on muscle pathology in Pompe disease have intensified and the role of defective autophagy has become a matter of concern. Some intriguing issues remained unsolved, for instance: why are the proximal muscles more affected than the distal muscles, and does the muscle fiber-type distribution play a role in disease severity, as might be concluded from studies performed in the mouse model of Pompe disease. The latter question can now be answered as the muscle fiber-type distribution does not seem to have a decisive impact on the clinical course of Pompe disease in humans (**chapter 3**). Thus, efforts to modify the fiber-type distribution do not seem useful, as

also already demonstrated in mice.[28] The former question about the preferential involvement of the proximal muscles was not specifically addressed in my studies. A thorough analysis of the clinical presentation of all of the 600 known neuromuscular disorders is beyond the scope of this thesis, but a brief review indicates that there seems to be resemblance in genes in both the distal myopathies as well as the proximal myopathies. However, the genetic programs leading to preferential involvement have remained unknown.

Attention should certainly be given to the role of autophagy in Pompe disease and how to possibly minimize autophagic build-up. Whether the autophagic build-up is a secondary effect due to lysosomal dysfunction or caused by more subtle metabolic changes is not entirely clear, but it seems reasonable to presume that it can be prevented by timely correction of the lysosomal acid α-glucosidase deficiency. With the current stage of art, ERT does not seem sufficiently effective to fully achieve this goal. Based on the current literature and personal experiences described in this thesis it is not to be expected that regular analysis of muscle biopsies are very helpful in determining the proper time of intervention with ERT in preclinical cases. ERT seems most effective when started in a very early stage of disease, when mildly affected adult patients may not demonstrate obvious muscle pathology.

With regard to the repair of pre-existing muscle damage, as possible adjunctive treatment in Pompe disease, it is relevant to mention that skeletal muscle has the ability to regenerate after traumatic injury and in diseases such as Pompe disease characterized by muscle damage.[29] Satellite cells are essential for the regeneration of muscle fibers. A recent study in Pompe knock-out (*GAA* -/-) mice has shown that the number of regenerating muscle fibers exceeds that of healthy mice. It is known from other myopathies that the pool of satellite cells has a limit.[30] Muscle damage starting early in life leads to the exhaustion of normal muscle repair mechanisms. With an eye on the future, more knowledge is required about the regenerative potential of the satellite cells in Pompe disease and the potential application of muscle stem cell supplementation as a mode to manage and treat patients with Pompe disease.

Residual disease and co-morbidities

The availability of ERT has changed patient's perspectives in terms of improved survival, muscle strength and pulmonary function. At the same time new clinical issues have arisen in terms of how to manage residual disease and co-morbidities. The issue counts most in classic infantile Pome disease. Now that the infants live much longer thanks to ERT problems such as hearing loss, speech difficulties and dysphagia, and an increased risk for pneumonia have been reported. Treated and untreated children and adults with Pompe disease have for instance,

increased aortic stiffness, and cerebral aneurysms may occur. Bone fractures can easily occur due to osteoporosis as described in this thesis. The patients' quality of life can be improved by paying proper attention to these less well known clinical features of Pompe disease, some of which have come to light only after the start of ERT. The co-morbidities may be related to the more known primary clinical features, for example the decreased BMD as consequence of the decreased muscle strength (*this thesis*). Since those primary clinical features should be corrected first in order to receive an effect on the secondary ones longer follow-up of ERT is required as shown by the lack of effect of ERT on BMD unless a increase in muscle strength described in this thesis.

Exercise training

Patients' fitness and physical functioning may be supported by treatments other than ERT or additional to ERT. In this thesis it was shown that the endurance, muscle strength and core stability of mildly affected non-classic patients improved when they followed a proper training program. Additionall, the patients experienced less fatigue and pain, and their mental health improved too. With some adaptations, the exercise program might also be helpful for children and more severely affected patients. The adaptations can involve modifications in length or intensity of each of the different parts of the program.

Final remarks

The work described in this thesis was undertaken to explore unknowns about Pompe disease with respect to i) muscle fiber-type distribution and muscle fiber-type-specific damage; ii) the BMD and the body composition of patients; and iii) the value of specific training programs for the management of Pompe disease. The studies were aimed to extract detailed information on muscle cell pathology and have illustrated the somewhat limited value of in depth analysis of muscle biopsies when it comes to explaining the cause of clinical diversity and providing a prognosis to affected non-classic patients. In classic infantile Pompe disease, however, the stage of muscle cell pathology holds pace with the age of untreated patients and is informative about the efficacy of ERT. Patients across the clinical spectrum of Pompe disease have a low BMD increasing their risk of bone fractures. Due to the low muscle mass of patients with Pompe disease FMI is increased which may predispose them for cardiovascular disease. Mildly affected non-classic patients respond well to the specific training program, which makes it worthwhile advertising these programs and developing adapted programs for more severely affected patients' clinical management.

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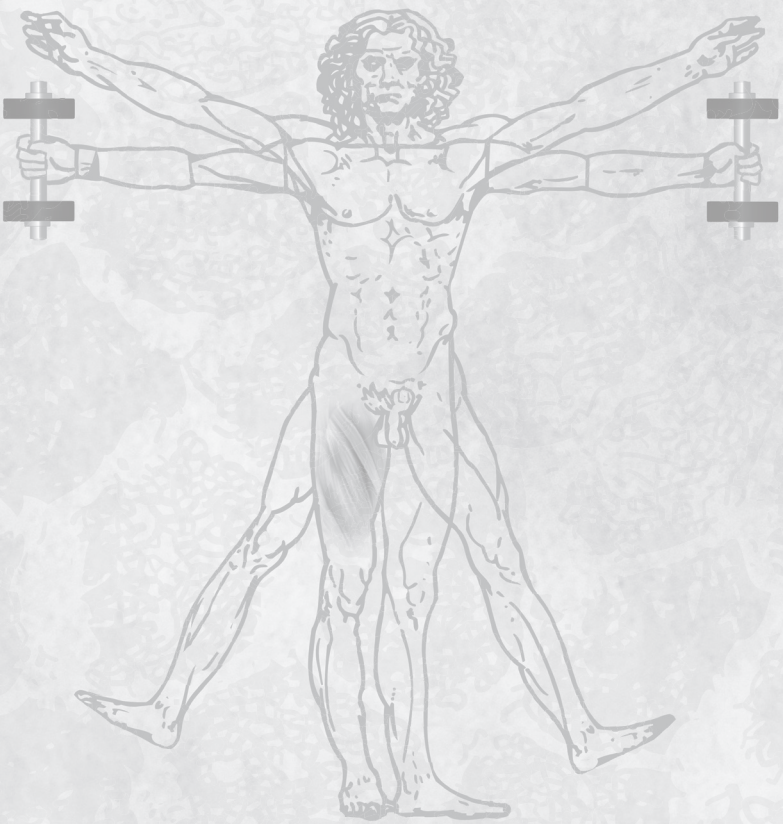
CHAPTER 9

Summary

Samenvatting



Summary

Pompe disease is an autosomal recessive lysosomal storage disorder, which is caused by a deficiency of lysosomal acid α-glucosidase leading to the accumulation of glycogen within lysosomes mainly in muscle cells. This ultimately results in the loss of muscle function and therefore Pompe disease can also be categorized as neuromuscular disorder. All patients suffer from muscle weakness, which can ultimately lead to wheelchair use and artificial ventilation.

Since its registration in 2006, enzyme replacement therapy (ERT) is the only treatment available for patients with Pompe disease. Although the availability of ERT has changed patient's perspectives in terms of improved survival, increased muscle strength and stabilisation of pulmonary function, not all patients respond equally well, and not all muscle damage and functional impairment is resolved.

The studies described in this thesis explore the muscle pathology in Pompe disease across the clinical spectrum, the interplay between skeletal muscle dysfunction and bone structure and the effect of enzyme therapy on it, as well as the use of exercise training to further improve patients' functioning.

In **Chapter 1** an introduction to Pompe disease is given. It provides background information about disease pathogenesis and pathophysiology, disease presentation and clinical spectrum, diagnosis and enzyme replacement therapy. Furthermore it numerates the current knowledge on the effects of exercise training in Pompe disease and other myopathies.

Chapter 2 presents a case of non-classic Pompe disease with a rather unusual clinical presentation of severe fatigue and myalgia prior to the development of limb-girdle weakness. In this special case fiber type involvement was restricted to the type 1 muscle fibers. With this case report we wanted to draw attention to the occurrence of fiber-type-specific pathology in Pompe disease, which was studied in detail in a larger group of patients in **Chapter 3**. Overall, patients with Pompe disease have the same muscle fiber-type distribution as healthy persons, except that patients in an advanced stage of disease seem to have a greater proportion of type 2x muscle fibers. As other researchers also noticed it is impossible to quantify the differences between the muscle biopsies of severely affected and less affected patients, despite the fact that there are clearly differences when visually inspected. These findings emphasize the

need for appropriate and sensitive technologies for the routine follow-up of patients and for investigating the effects of ERT.

The focus of **Chapter 4** and **Chapter 5** is on bone mineral density (BMD) and body composition. In **Chapter 4** the BMD was systematically assessed in a cohort of 46 patients, before the start of ERT using DXA technology. Low BMD, a possible indicator of osteoporosis as co-morbidity, was found to be common in patients with Pompe disease. At particular risk of developing this trait are children and patients who are wheelchair-bound and ventilator-dependent. However, in ambulant patients we also found low BMD. The low BMD of patients with Pompe disease correlates with decreased proximal muscle strength. The outcome of this study signifies the importance of screening for decreased BMD in all affected children, wheelchair-bound and ventilator-dependent adults and in all patients with progressive loss of muscle strength. With ERT eliciting a positive effect on skeletal muscle strength, it might also improve the BMD and influence body composition. Therefore, we investigated in **Chapter 5** the effect of ERT on the BMD and body composition of 35 adult patients with Pompe disease. We discovered, that they had a lower lean body mass than healthy individuals; which is most likely due to their decreased muscle mass. Classification of patients according to either body mass index (BMI) or fat mass index (FMI) gave different results: three patients were classified as obese according to the BMI in contrast to 13 patients according to the FMI. In clinical practice the fat mass index (FMI) should be used preferably, since calculation of the BMI underestimates the fat mass of Pompe patients. No clinically meaningful improvement in BMD nor body composition was observed within 2-3 years of enzyme therapy despite an increase in muscle strength.

In **Chapter 6** and **Chapter 7** exercise training in addition to ERT is explored in order to improve patients' fitness and physical functioning. A standardized and well-structured exercise intervention program combining aerobic, resistance and core stability exercises was followed by 25 patients three times a week for 12 weeks. Two patients did not complete the training program because it was too time-consuming for them. This left 23 patients, who successfully completed the study. In **Chapter 6** we evaluate the effects of such a regime on endurance, muscle strength and function, core stability, and body composition. In **Chapter 7** we present the effect of the combined training program on pain, fatigue, activity and participation. We show that the program is feasible and can be performed safely by mildly affected patients with Pompe disease. As intended, such training helps to improve endurance, muscle strength, muscle function and core stability. The training program significantly improves fatigue and

pain, and tends to improve mental health. It does not affect body composition, the quality of motor function nor the volume of physical activity. The observed improvements in pain and fatigue are not determined by aerobic fitness, muscle strength or core stability. This training program thus seems to offer added value for Pompe patients receiving long-term ERT.

Chapter 8 discusses the main findings, their clinical implications for present day practise, and contains suggestions for follow-up research.

Samenvatting

De ziekte van Pompe is een autosomaal recessief overerfende lysosomale stapelings-ziekte die veroorzaakt wordt door een gebrek aan het enzym zure α-glucosidase. Tekort aan dit enzym leidt tot stapeling van glycogeen in de lysosomen van hoofdzakelijk spiercellen, wat uiteindelijk leidt tot verlies van spierfunctie. Hierom kan de ziekte van Pompe ook ingedeeld worden bij de neuromusculaire ziekten. Patiënten met de ziekte van Pompe hebben spierzwakte waardoor velen van hen uiteindelijk rolstoelgebonden en beademingsbehoeftig raken.

Sinds 2006 is er enzymtherapie; vooralsnog de enige behandeling die beschikbaar is voor patiënten met de ziekte van Pompe. De introductie van enzymtherapie heeft geleid tot levensverlenging, verbetering van de spierkracht en tot stabilisatie van de longfunctie van de patiënten, maar niet alle patiënten reageren even goed op de behandeling en niet alle spierschade en functieverlies wordt hersteld.

De studies die in dit proefschrift beschreven staan onderzoeken de spierpathologie van patiënten met de ziekte van Pompe; bij heel jonge kinderen zowel als bij volwassenen , de wisselwerking tussen spierfunctie en de kwaliteit van bot, en de effecten van enzymtherapie hierop. Ook wordt de mogelijkheid onderzocht het fysiek functioneren van patiënten te verbeteren door middel van training

In **Hoofdstuk 1** wordt de ziekte van Pompe geïntroduceerd. Dit hoofdstuk geeft achtergrond-informatie over de pathogenese en de pathophysiologie, de klinische kenmerken, de diagnose en de behandeling met enzymtherapie. Verder wordt de kennis van de effecten van training bij ziekte van Pompe en andere spierziekten beschreven.

In **Hoofdstuk 2** wordt een casus gepresenteerd. Deze patiënt, met beginnende symptomen van de ziekte van Pompe op volwassen leeftijd, presenteerde zich anders dan gebruikelijk met klachten van ernstige vermoeidheid, nog voordat klachten van spierzwakte zich ontwikkelden. In het spierbiopt van de patiënt waren alleen de type 1 spiervezels aangedaan. Door het beschrijven van deze casus wilden wij de spiervezelspecifieke pathologie bij de ziekte van Pompe onder de aandacht brengen. Deze pathologie werd in meer detail bestudeerd in **Hoofdstuk 3**. Daarbij bleek dat patiënten met de ziekte van Pompe geen andere spiervezelverdeling hebben dan gezonde volwassenen, met uitzondering van patiënten in een meer ver gevorderd stadium van de ziekte. Deze laatste patiënten lijken meer type 2x spiervezels te hebben. Ondanks de

microscopisch zichtbare verschillen tussen de spierbiopten van meer en minder aangedane patienten, slaagden wij er niet, in het verschil tussen ernstig aangedane en minder ernstig aangedane spieren te patiënten kwantificeren. Andere onderzoekers hebben dit probleem eerder al ervaren en deze studie benadrukt de behoefte aan geschikte en gevoelige technieken om spierschade kwantitatief te meten, zodat patiënten in de loop van de tijd goed gevolgd kunnen worden en ook om de effecten van ERT te kunnen bestuderen.

In **Hoofdstuk 4** en **Hoofdstuk 5** wordt aandacht besteed aan botdichtheid en lichaamssamenstelling. **Hoofdstuk 4** onderzoekt systematisch, middels DEXA-scans, de botstatus van 46 patiënten, die nog niet werden behandeld met ERT. Patiënten met de ziekte van Pompe blijken vaak een lage botdichtheid te hebben en lopen dus de kans op osteoporose als co-morbiditeit. Dit geldt vooral voor kinderen, rolstoelgebonden- of beademingsbehoeftige patiënten. De lage botdichtheid bleek samen te hangen met verminderde kracht in de proximale spieren. Deze studie geeft aan dat het belangrijk is om te screenen voor een verlaagde botdichtheid bij alle kinderen met de ziekte van Pompe, alle rolstoelgebonden en beademingsbehoeftige volwassen patiënten en alle patiënten die in spierkracht achteruitgaan. Aangezien ERT de spierkracht verbetert kan het mogelijk ook de botdichtheid verbeteren. Daarom wordt in **Hoofdstuk 5** het effect van ERT op de lichaamssamenstelling en botdichtheid van 35 volwassen patiënten met de ziekte van Pompe onderzocht. Voor aanvang van de behandeling bleken deze patiënten een lagere vetvrije massa dan gezonde volwassenen te hebben. Dit komt waarschijnlijk doordat patiënten minder spiermassa hebben. Bij het indelen van patiënten in groepen volgens de body mass index (BMI) en de fat mass index (FMI) bleek dat 3 patiënten obees waren op basis van hun BMI, terwijl 13 patiënten obees waren volgens hun FMI. Omdat de BMI dus de vetmassa van patiënten lijkt te onderschatten, is het beter om in de kliniek gebruik te maken van de FMI. Ondanks een verbetering van de spierkracht werd geen klinisch relevante verbetering van botdichtheid of lichaamssamenstelling gevonden na 2 jaar behandeling met ERT.

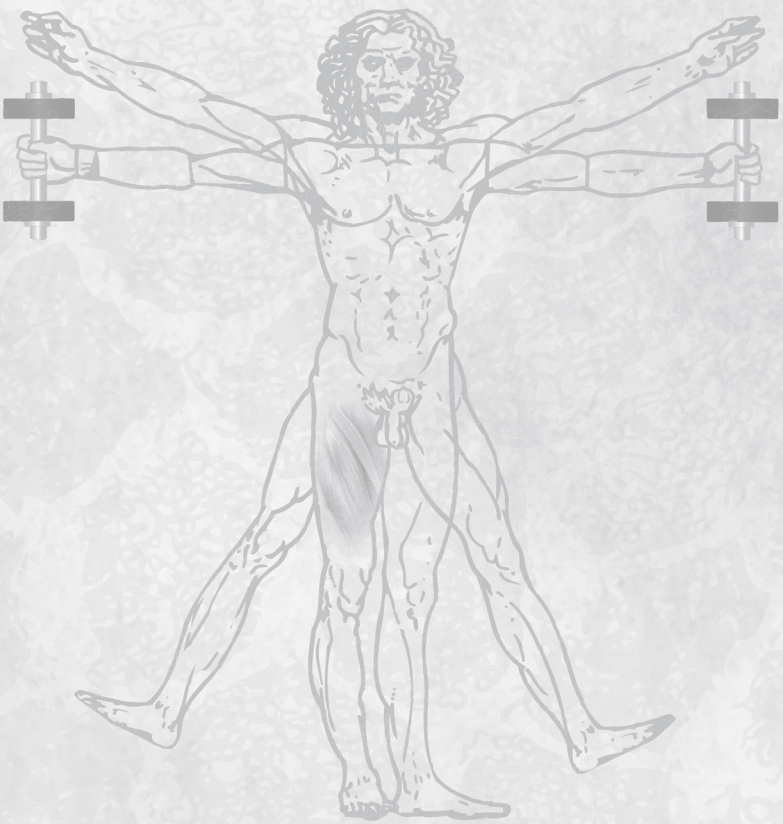
De rol van training naast de behandeling met enzymtherapie met als doel het verder verbeteren van de fitheid en het fysiek functioneren van patiënten wordt onderzocht in **Hoofdstuk 6** en **Hoofdstuk 7**. Vijfentwintig patiënten volgden drie keer per week gedurende 12 weken een gestandaardiseerd en gestructureerd trainingsprogramma bestaande uit duur-, kracht- en rompstabiliteitstraining. Twee patiënten vielen af, omdat het trainingsprogramma te veel van hun tijd in beslag nam. In **Hoofdstuk 6** worden de effecten van het programma op uithoudingsvermogen, spierkracht en spierfunctie, rompstabiliteit en lichaamssamenstelling

onderzocht. De effecten op pijn, vermoeidheid, activiteitenniveau en participatie worden in **Hoofdstuk 7** gepresenteerd. Het beschreven trainingsprogramma is voor mild aangedane patiënten met de ziekte van Pompe haalbaar om uit te voeren en is ook veilig gebleken. Zoals bedoeld helpt het trainingsprogramma het uithoudingsvermogen, de spierkracht, de spierfunctie en de rompstabiliteit te verbeteren. Patiënten waren minder moe en hadden minder pijn aan het einde van het onderzoek. We konden echter niet achterhalen of het ervaren van minder vermoeidheid en pijn kwam door verbeteringen in het uithoudingsvermogen, de spierkracht of de rompstabiliteit. Ook de mentale gezondheid leek te verbeteren. Het programma had geen effect op de lichaamssamenstelling, de kwaliteit van beweging of de mate van fysieke activiteit. Het volgen van dit trainingsprogramma bestaande uit duur-, kracht- en rompstabiliteitstraining naast de behandeling met ERT lijkt dus toegevoegde waarde te hebben voor mild aangedane patiënten met de ziekte van Pompe.

Hoofdstuk 8 bediscussieert de belangrijkste bevindingen die in mijn proefschrift beschreven staan, hoe ze in praktijk gebracht kunnen worden en gebruikt kunnen worden voor vervolgonderzoek.

CHAPTER 10

List of abbreviations
Dankwoord
Curriculum Vitae
Publications
PhD portfolio



List of abbreviations

6MWT	6-minute walk test
ALT	Alanine aminotransferase
ANA	Antinuclear antibodies
AST	Aspartate aminotransferase
BMD	Bone mineral density
BMI	Body mass index
BPM	Beats per minute
CHO	Chinese hamster ovary
CK	Creatine kinase
COX	Cytochrome oxidase
DM	Dermatomyositis
DSHB	Developmental Studies Hybridoma Bank
DXA	Dual energy X-ray absorptiometry
ECG	Electrocardiography
ECLIA	electrochemiluminescence
EMA	European Medicines Agency
EMG	Electromyography
ERT	Enzyme replacement therapy
ESR	Erythrocyte sedimentation rate
FDA	US Food and Drug Administration
FM	Fat mass
FMI	Fat mass index based on fat mass/height^2
FN	Femoral neck
FSHD	Facioscapulohumeral muscular dystrophy
FSS	Fatigue Severity Scale
FVC	Forced vital capacity
GAA	gene coding for acid α-glucosidase
HHD	Hand-held dynamometry
HR	Heart rate
IBM	Inclusion body myositis
ICF	International classification of functioning, disability and health

LBM	Lean body mass/height^2
LDH	Lactate dehydrogenase
LGMD	Limb-girdle muscular dystrophy
LS	Lumbar spine
MARS	Muscle assessment and rating scores
MCS	Mental component summary
MD	Muscular dystrophy
MHC	Myosin heavy-chain
MMT	Manual muscle testing
MRC	Medical Research Council
mtDNA	mitochondrial DNA
n	Number
NADH	Nicotamide adenine dinucleotide
OMIM	Online Mendelian Inheritance in Man
ORO	Oil red O
PCS	Physical component summary
PM	Polymyositis
QMFT	Quick motor function test
QMT	Quantative muscle testing
QoL	Quality of life
RER	Respiratory exchange rate
rhGAA	recombinant human acid α-glucosidase
R-PAct	Rash-built self-reported Pompe-specific Activity Scale
SD	Standard deviations
SDH	Succinate dehydrogenase
SF-36	Medical Outcomes Study 36-item short-form health survey
TB	Total body
TSH	Thyroid-stimulating hormone
VO_2 PEAK	Peak oxygen uptake
VT	Ventilatory treshold
WMAX	Maximum workload capacity

Dankwoord

Bij het begin van je promotie droom je hierover, op momenten dat het tegen zit schrijf je het in gedachten en nu het moet is het nog niet klaar…. Zou dat dan toch komen omdat dit het enige onderdeel is van het hele promotietraject wat ik moet doen zonder de hulp van alle anderen, die ik hier zo graag wil bedanken?

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About the author

Linda van den Berg was born on May 9th, 1979 in Bergen op Zoom, the Netherlands as a daughter of Kees and Ria van den Berg and little sister of Robin. In 1997 she graduated from the Mollerlyceum (pre-university education) in Bergen op Zoom, and started studying Health Sciences at Maastricht University. During one year of her study she was chairman of Dynamis (student association). After finishing her research project on exercise training in type II diabetes, she obtained her degree in Human Movement Sciences in 2002. One year early, in 2001, she started her medical training at the Erasmus University Rotterdam (nowadays Erasmus MC University Medical Center). In August 2007 she obtained her medical degree and started as a PhD-student at the Center for Lysosomal and Metabolic Diseases at Erasmus MC University Medical Center under supervision of Prof. dr. A.T. van der Ploeg and dr. A.J.J. Reuser. During her PhD-training she worked as Movement Consultant and Club Doctor for the youth teams of the soccer club, Sparta Rotterdam. In January 2013 she started her trainingship in Sports Medicine in MC Haaglanden in Leidschendam under supervision of R.F. van Oosterom. Linda lives in Tholen with her husband Jules en daughter Juliette.

Publications

Van den Berg LEM, Zandbergen AA, van Capelle CI, de Vries JM, Hop WC, van den Hout JMP, Reuser AJJ, Zillikens MC, van der Ploeg AT. Low bone mass in Pompe disease: muscular strength as a predictor of bone mineral density. *Bone* 2010; **47**: 643-649

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Van den Berg LEM, Drost MR, Schaart G, de Laat J, van Doorn PA, van der Ploeg AT, Reuser AJJ. Muscle fiber-type distribution, fiber-type-specific damage, and the Pompe disease phenotype. *J Inherit Metab Dis* 2013; **36**: 787-794

Güngör D, de Vries JM, Brusse E, Kruijshaar ME, Hop WC, Murawska M, **van den Berg LE**, Reuser AJJ, van Doorn PA. Enzym ereplacement therapy and fatigue in adults with Pompe disease. *Mol Genet Metab* 2013; **109**: 174-178

Favejee MM, **van den Berg LEM**, Kruijshaar ME, Wens SCA, Praet SFE, Pijnappel WWM, van Doorn PA, Bussmann JBJ, van der Ploeg AT. Exercise training in adults with Pompe disease: the effects on pain fatigue, and functioning. *(submitted)*

Van den Berg LEM, Favejee MM, Wens SCA, Kruijshaar ME, Praet SFE, Reuser AJJ, Bussmann JBJ, van Doorn PA, van der Ploeg AT. Safety and efficacy of exercise training in 23 adults with Pompe disease receiving enzyme therapy. *(submitted)*

Van den Berg LEM, Kruijshaar ME, Zillikens MC, Karstens FPJ, Wens SCA, van den Hout JMP, Pijnappel WWM, van der Ploeg AT. Effects of 2 years enzyme replacement therapy on bone mass and body composition in 35 adults with Pompe disease. *(in preparation)*

PhD Portfolio

SUMMARY OF PHD TRAINING AND TEACHING ACTIVITIES

Erasmus MC Department:	**Pediatrics, Center for Lysosomal and Metabolic Disease**
Research School:	**Molecular Medicine Postgraduate School**
PhD period:	**2007-2014**
Promotor:	**Prof.dr. A.T. van der Ploeg**

	Year	Workload (ECTS)
General academic skills		
– Biomedical English Writing and Communication	2009-2010	4.0
– Research Integrity	2009	2.0
– Basiscursus regelgeving en management (BROK)	2009	0.9
Research skills		
– Biweekly journal club, Center for Lysosomal and Metabolic Diseases	2007-2012	1.5
– Weekly or biweekly research meeting, Center for Lysosomal and Metabolic Diseases	2007-2012	3.0
– Reviewing paper for peer reviewed journal	2010	0.3
In-depth courses		
– International postgraduate course on lysosomal storage disorders, Nierstein, Germany	2007	1.2
– 2nd European symposium Steps Forward in Pompe disease, Nice, France	2007	0.5
– International workshop on lysosomal storage disorders, Brussels, Belgium	2007	0.5
– 5th Symposium on lysosomal storage disorders, Paris, France	2007	0.5
Seminars and workshops		
– Talent class Marketing yourself effectively, Netherlands Organisation for Scientific Research (NWO)	2009	0.3
– Talent class Oral Presentation, Netherlands Organisation for Scientific Research (NWO)	2010	0.3

	Year	Workload (ECTS)
Presentations and international conferences		
– Sophia Research Day, Rotterdam, The Netherlands (poster presentation)	2008	0.5
– 3rd European symposium Steps Forward in Pompe disease, Munich, Germany (poster presentation)	2009	0.5
– Spierziektendag Vereniging Spierziekten Nederland (VSN), Zoetermeer, The Netherlands (oral presentation)	2009	1.0
– Najaarsvergadering Erfelijke Stofwisselingsziekten in het Nederlands Taalgebied, Driebergen, The Netherlands (oral presentation)	2009	1.0
– 11th International Congress of Inborn Errors of Metabolism, San Diego, California, USA (poster presentation)	2009	0.5
– Sophia Research Day, Rotterdam, The Netherlands (oral presentation)	2009	1.0
– MPS patient day, Amersfoort, The Netherlands (oral presentation)	2009	1.0
– 4th European symposium Steps Forward in Pompe disease, London, United Kingdom (poster presentation)	2010	0.5
– AGSD-UK Annual Meeting, London, United Kingdom (oral presentation)	2010	1.0
– 7th Pompe disease expert day, Rotterdam, The Netherlands (oral presentation)	2010	1.0 1.0
– 8th Pompe disease expert day, Rotterdam, The Netherlands (oral presentation)	2010	
– 5th European symposium Steps Forward in Pompe disease, Budapest,Hungary (poster presentation)	2011	0.5
– 4th International Congress of Myology, Lille, France (poster presentation)	2011	0.5
– AMDA/IPA Patient and Scientific Conference, San Antonio, Texas, USA (oral presentation)	2011	1.0
– AGSD-UL Annual Meeting, Telford, United Kingdom (oral presentation)	2011	1.0

	Year	Workload (ECTS)
– Spierziektendag Vereniging Spierziekten Nederland (VSN), Lunteren, The Netherlands (oral presentation)	2011	1.0
– 6th European symposium Steps Forward in Pompe disease, Berlin, Germany (poster presentation)	2012	0.5
– Spierziektendag Vereniging Spierziekten Nederland (VSN), Veldhoven, The Netherlands (oral presentation)	2012	1.0
– Webinar AMDA	2012	1.0
Teaching activities		
– Supervising Master's thesis	2007-2008	3.0
– Supervising student 'laboratory skills and introduction to research'	2008-2009	1.0
– Introduction to Pompe disease for physiotherapists	2011	1.0
Other		
– Clinical work Division of Metabolic Diseases	2007-2008	