CHAPTER I

General Introduction.
Duchenne muscular dystrophy (DMD) is an X-linked recessive neuromuscular disorder clinically characterised by progressive muscle weakness starting in childhood and leading to death around the age of 20 years.\(^1\) With an estimated birth prevalence of 1 in 4200 live born males, it is one of the most frequent neuromuscular disorders.\(^2\)

**Clinical signs and symptoms**

Initial complaints in most boys with DMD are an abnormal gait with toe walking, frequent falls and difficulties with climbing stairs. There is often a history of delayed achievement of motor milestones. The mean age at diagnosis is between 4 and 5 years\(^3,4,5\) when proximal muscle weakness is sufficiently severe to produce a waddling gait and problems with rising from the floor.

On physical examination one may find mild proximal muscle weakness in the lower limbs with a Gowers sign, shortened ankle tendons and tendon reflexes that are still present. There is compensatory lumbar hyperlordosis, which disappears when the child is sitting. The calf muscles are large and feel quite firm or even rubbery due to replacement of muscle fibres by fibrofatty tissue.

Besides muscle weakness mental impairment can be present in DMD.\(^6\) Approximately 33% of the patients has an intelligence quotient - score below 75.\(^7\)

Due to the progressive nature of the muscle disease, there is a linear decline in muscle strength making the children wheelchair bound at a mean age of 9,5 years.\(^8\)

Scoliosis is a frequent complication that may require surgical intervention. Nocturnal hypoventilation may be the first sign of respiratory insufficiency. If present, the child awakens frequently, is afraid of sleep and has headaches in the early morning. Signs of cardiomyopathy are already present in 25% of patients under 6 years, up to 59% in those between ages 6 and 10, without giving any symptoms.\(^9\) Eventually, DMD patients die from respiratory insufficiency due to intercurrent infection or aspiration, or from cardiac arrhythmia. Patients who do not receive artificial ventilation from an early stage die at a mean age of 19 years, those who are ventilated at a mean age of 25 years.\(^1\)

**Pathophysiology**

DMD is caused by mutations of the dystrophin gene which is located on the short arm of the X-chromosome (Xp21).\(^10\) The protein product, called dystrophin, is situated at the sarcolemma in muscle fibres\(^11,12\) and is part of the dystrophin-glycoprotein-complex
The DGC provides a structural link between the actin cytoskeleton and the extracellular matrix, thereby stabilising the sarcolemma during cycles of muscle contraction and relaxation. However, the precise mechanism by which dystrophin deficiency causes the destruction of muscle fibres is still unknown.

Diagnosis

The diagnosis of DMD is based on clinical signs and symptoms and confirmed by raised serum concentration of creatine kinase (CK), absence of dystrophin in muscle biopsy, and the finding of a mutation in the dystrophin gene. In all DMD patients serum CK is raised from birth onwards and exceeds at least 10 times the normal upper limit. After 5 years of age the concentration slowly declines. The muscle biopsy shows abnormalities typical of muscular dystrophy such as necrosis and attempted regeneration of individual muscle fibres, increased variability of muscle fibre diameter with both hypertrophic and small fibres, and central nuclei. (Figure 1). In an end-stage biopsy, almost the entire muscle is replaced by fibrofatty tissue. To confirm the clinical diagnosis immunohistochemical analysis of the muscle biopsy is usually performed. If this shows complete absence or severe reduction of dystrophin with only an occasional fibre (less than 5%) staining positively, further genetic analysis is performed. DMD must be differentiated from Becker muscular dystrophy (BMD), which is caused by mutations in the same gene but has a milder phenotype.

Genetics

Mutations in the dystrophin gene can cause DMD or BMD. This is explained by the reading frame hypothesis which states that mutations that maintain the reading frame (in-frame mutations) generally result in abnormal but partly functional dystrophin and usually cause BMD. In DMD patients however, mutations disrupt the reading frame (frame-shift mutations), which eventually leads to dystrophin deficiency. In DMD and BMD, 65% of the pathogenic changes are large partial deletions usually deleting multiple exons and 5% are partial duplications of one or more exons. Both can be detected by quantitative multiplex PCR or Southern blot analysis. These partial deletions/duplications are clustered in two hot-spot regions of the gene, one proximal region comprising exons 2-20 and one more distal region comprising exons 45-53 in the gene. Part of the remaining 35% of pathogenic changes are most likely explained by small duplications or deletions that are often missed by the
techniques mentioned earlier. These small changes can be detected, however, by one of many different pre-screening methods or direct sequencing. One of the most sensitive methods is denaturing gradient gel electrophoresis (DGGE) which has a detection rate of almost 100%. 22,23

Therapy

There is no cure for DMD. Treatment goals are to maintain function, prevent contractures, and provide psychological support to the child and its family. Main efforts should be directed towards keeping these children standing and walking as long as possible. Passive stretching exercises, use of splints to maintain the feet in a neutral position during the night, and use of long-leg braces for walking are important in this respect. Scoliosis can not be prevented and, if progressive, surgical correction is the only effective way to straighten the spine. Steroids do have a beneficial effect on muscle force and muscle function, but their use is not yet generally accepted because of uncertainties about both the positive and negative effects on the long run. Other treatments aiming at the correction of the gene defect itself are not yet available for clinical use.

Figure 1. Muscle biopsy from a 5 years old DMD patient.
Outline of the thesis

This thesis consists of two parts. The first part (Chapters 2-5) describes the results of diagnostic studies concerning muscle force, functional ability, calf circumference and ultrasonographic findings in DMD patients and healthy controls. Establishing reference values is important for several reasons such as for diagnostic purposes, for determining the extent and progression of the disease, and for evaluating possible effects of certain treatments. The second part of this thesis (Chapter 6-7) deals with the issue of prednisone therapy in DMD.

Chapter 1, gives a short overview of the clinical, pathological and genetic aspects of DMD. In Chapter 2, reference values for maximum muscle force in normal children are described. In Chapter 3, these reference values are applied in ambulant DMD patients to assess the extent of muscle weakness and functional impairment. In Chapter 4, the extent of calf enlargement in ambulant DMD patients is compared with that in healthy controls.

In Chapter 5, a complete set of quantitative ultrasonographic findings in biceps and quadriceps muscles of healthy children is presented. These data are applied in children with proven neuromuscular disease to evaluate their additional diagnostic value.

In Chapter 6, a review of all aspects considering steroid treatment in DMD is given, based on the available literature. In Chapter 7, the results of a randomised controlled trial of the effects of prednisone on muscle function and the quality of life in ambulant DMD patients are presented.

Finally, a summary and conclusions concerning this thesis are given.
Reference List


