Summary

Carriers of Duchenne (DMD) and Becker (BMD) muscular dystrophy may show muscle weakness or dilated cardiomyopathy (DCM). Earlier studies only focussing on skeletal muscle involvement have been done before DNA analysis was possible. Therefore, we undertook a cross-sectional study in a population of definite carriers to estimate the proportion and to assess the clinical profile of symptomatic carriers. Furthermore, we investigated a possible correlation between genotype and phenotype. DMD and BMD carriers, aged 18-60 years, were traced through the files of the central register kept at the department of Human Genetics in Leiden, Netherlands. In a follow-up study in 1994, we examined the progression of cardiac abnormalities in 27 patients with BMD.

In **Chapter one** the results of the assessment of muscle weakness and severe cardiac abnormalities are described to detect the proportion of symptomatic carriers in definite carriers.
Hundred and twenty-nine carriers (85 DMD, 44 BMD) participated which was 48% of the carrier file. In 90 females from 52 families (70%) 37 different mutations were found. Twenty-four carriers (19%) were detected by linkage analysis and 15 carriers (12%) were obligate. Twenty-eight carriers (22%; DMD 26%, BMD 13%) were symptomatic. Twenty-two carriers (17%; DMD 19%, BMD 14%) had muscle weakness, varying from mild to moderately severe. In 18 women (82%) muscle weakness was predominantly asymmetric with a predilection for the shoulder girdle/upper arms in nine carriers (41%). In five (23%) only the pelvic girdle/upper legs were involved. No severe muscle weakness was found. Seven carriers (5%) had dilated cardiomyopathy, of whom one had concomitant muscle weakness. No correlation was found between genotype and muscle weakness. It was concluded that clinical manifestation of muscle weakness, dilated cardiomyopathy, or both can be found in about one-fifth of carriers of DMD and BMD.

Chapter two presents the results of an extensive cardiological evaluation in 129 DMD/BMD carriers. Investigations included full medical history, physical examination, electrocardiographic (ECG) and two-dimensional and M-mode echocardiographic examination. Sixty-one carriers (47%; DMD 51%, BMD 41%) had ECG changes. Forty-seven women (36%; DMD 41%, BMD 27%) had at least one abnormality as is usually found in male patients. Echocardiographic examination was abnormal in 47 carriers (36%; DMD 38%, BMD 34%). DCM was found in seven DMD carriers (8%), and in none of BMD carriers. In addition, 23 women (18%) had left ventricle dilatation (DMD 19%, BMD 16%). Other echocardiographic abnormalities included right ventricle and left and right atrial dilation, wall motion abnormalities, and systolic or diastolic dysfunction. Only 38 % of carriers

had a completely normal investigation of the heart. We found no association between mutation type or site and dilated cardiomyopathy. The study underlines that cardiac involvement is part of the spectrum of dystrophinopathies.

Chapter three evaluates the utility of dystrophin analysis (immunohistochemistry and Western blot analysis) of muscle tissue in 50 DMD/BMD carriers in order to assess possible associations between clinical phenotype (muscle weakness, dilated cardiomyopathy). We have also investigated the proportion of dystrophin abnormalities in asymptomatic carriers to evaluate the value of dystrophin analysis for carrier detection. Fifty carriers underwent a muscle biopsy: 17 with muscle weakness, five with dilated cardiomyopathy, five with cramps and 23 asymptomatic carriers. In 16 carriers (32%) dystrophin abnormalities (immunohistochemistry or Western blot) were detected (9 DMD, 7 BMD): five carriers with muscle weakness, nine asymptomatic carriers and two carriers with cramps. Immunohistochemistry was abnormal in 10 carriers: three carriers with muscle weakness, one carrier with cramps and six asymptomatic carriers. Therefore, dystrophin analysis can not be used as a diagnostic test for symptomatic carriers. Even in carriers with muscle weakness immunohistochemistry is most often normal. Immunohistochemical staining revealed dystrophin negative fibers in 26% of asymptomatic carriers. Possibly, this can be used in females with a positive family history for DMD/BMD in which DNA or linkage analysis is not feasible.

In **chapter four** the course of cardiac involvement in 27 previously reported patients with Becker Muscular Dystrophy (BMD), originating from nine kindreds is described. The mean follow-up period was 12.5 years.
Seventeen patients (63%) showed progression of cardiac abnormalities. Electrocardiographic abnormalities progressed from 44% to 71%. Dilated cardiomyopathy (DCM), with or without congestive heart failure, was now present in nine patients (33%) as compared to 15% in the previous study. In addition, 22% developed borderline echocardiographic abnormalities. Six patients (22%) became symptomatic and four patients died in congestive heart failure. In all families cardiac abnormalities were found. There was no association between DCM and mutation type or site. Despite equal functional motor ability, there was a considerable intrafamilial variability regarding cardiac involvement, even in brother pairs.
We have concluded that cardiac abnormalities are the rule and not the exception in BMD and are progressive over time. Left ventricular dilation may begin at any moment in the course of BMD and the rate of progression is unpredictable. A substantial part of patients will develop an incapacitating and life threatening DCM.

Chapter five reports on a study that has tested cardiac specific markers in BMD/DMD carriers for diagnosing non-ischemic myocardial damage. At present Troponin T and Troponin I are presumed to be the most cardiac specific markers. Laboratory investigations in 129 DMD/BMD carriers consisted of measurement of serum creatine kinase (CK), and aspartase aminotransferase (ASAT) activity, CK-MB-mass, cardiac Troponin T (cTnT) and cardiac Troponin I (cTnI).
Serum CK was raised in 45% of carriers (DMD 53%, BMD 30%), and these percentages are lower than in earlier studies. Twenty-two cases had an elevated CK-MB, but none demonstrated a CK-MB/CKTOTAL ratio above 6%. No elevation of cTnI was found and only one DMD carrier with borderline echocardiographic abnormalities had slightly increased cTnT. In conclusion, cTnT and cTnI elevation is very rare (cTnT) or does not occur (cTnI) in DMD/BMD carriers. They can not be used as a marker of non-ischemic myocardial damage.

In **chapter six** the most important findings described in previous chapters are discussed. We comment on the severity and significance of cardiac and skeletal muscle symptoms. We argue the need for screening for severe cardiac abnormalities among carriers. Furthermore, possible therapies for dilated cardiomyopathy are discussed. Lastly, the usefulness of dystrophin analysis of muscle tissue for carrier detection is questioned.