**BACKGROUND**

Duchenne muscular dystrophy (DMD) and its murine model, mdx, are characterized by Ca^{2+} induced muscle damage, fibrosis and muscle weakness. Furthermore, DMD patients have distorted dentofacial morphology which could be a result of changed masticatory mechanics due to muscular dysfunction.

**AIM**

Examination of the expression levels of myosin heavy chain (MHC)-isoforms and morphological abnormalities in head muscles of control and mdx mice.

**MATERIAL AND METHODS**

- **Real-time RT-PCR:** Total RNA (200 ng) was reverse transcribed using random hexamers. The cDNA was amplified with specifically designed probes and primers (Applied Biosystems) using an ABI 5700 sequence detection system (Applied Biosystems). All values are given in relation to the mRNA of 18S rRNA.
- **Morphology:** For staining cryosections were prepared and either stained with hemalaun / eosin for the visualisation of nuclei or stained with Sirius Red for the visualisation of collagen. Image analysis of up to 100 muscle fibres in each section was performed.
- **Western blot analysis:** Samples from masticatory muscles were subjected to SDS gels, transferred to nitrocellulose membranes and incubated with antibodies against skeletal myosin (clone NOQ7.5.4D and MY-32, Sigma; dilution 1:3000). Secondary HRP-conjugated goat anti-mouse IgG were used at a 1:5000 dilution. Visualization of bound antibodies was achieved with ECL Plus (Pierce).

**RESULTS**

**Fig.2 (A)** Bar histograms representing the mean muscle fibre diameters of age-matched control and mdx mice. Means ± S.E.M. are given in all cases. Stars indicate significant differences: ***) p < 0.005, unpaired t-test.

**Fig.3 (A)** Sirius Red stained cryo-sections from control and mdx mice showing the accumulation of collagen in dystrophic muscles. (B) Bar histograms representing the percentage of collagen expression of age-matched control and mdx mice. Means ± S.E.M. are given in all cases. Stars indicate significant differences **) p < 0.01; ***) p < 0.005, unpaired t-test. MAS, masseter; TEM, temporal; SOL, soleus; TON, tongue.

**Fig.4** Western blot analyses of fast (A; clone MY-32) and slow skeletal myosins (B; clone NOQ 7.5.4D) in SOL, TEM, MAS and TON of 100d old control and mdx mice. As loading control every membrane was stripped and incubated with a monoclonal anti-α-actinin antibody (clone AT6/172). (C) Quantitative analyses of Western blots as shown in A-B. Protein bands were evaluated using the GelScan 5.2 software (Serva, Germany). The ratio ± S.E.M. between mean optical densities (mod) of control and mdx mice are given in all cases.

**Fig.5** Levels of MyHC transcripts were determined by Real-time RT-PCR using RNA preparations from MAS (A), TEM (B), TON (C) and SOL (D). Samples were from 100d old mdx mice (dashed boxes) and control mice (black boxes). The mRNA levels of myosin heavy chains are given in relation to that of 18S rRNA. The observed down regulation of the MyHC IIx and IIb isoforms in mdx masticatory muscles are differentially affected by the disease process compared to hind limb muscles.

**CONCLUSION**

- The mdx mouse model could be useful for further investigations to study changes in the masticatory system of DMD patients.
- Mdx masticatory muscles are differentially affected by the disease process compared to hind limb muscles.
- The observed down regulation of the MyHC IIX and IIb isoforms in mdx mice may be responsible for the functional misbalance of masticatory muscles in DMD and could be causing morphological changes.