Levator palpebrae superioris fibre size in normals and patients with congenital ptosis

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ARTICLE

Abstract

A study of the histochemical staining of levator palpebrae superioris (LPS) was undertaken to measure the muscle fibre size in normal individuals and in patients with congenital dystrophic ptosis in order to see whether there is an alteration in muscle fibre size in congenital ptosis. Eight LPS specimens were obtained: 4 from normal patients (3 from normal levator divided in a bilateral brow suspension procedure and 1 from an exenteration specimen) and 4 from levator resection procedures for treatment of unilateral congenital ptosis. Cryostat sections from these specimens were stained histochemically to reveal muscle fibre types. The orthogonal diameters were measuring using a computer-generated program and the two groups compared using the Mann–Whitney *U*-test. No statistically significant difference in muscle fibre diameter was found between normals and patients with congenital ptosis. There was also no change in the distribution or range of muscle fibre diameter in patients with congenital ptosis. Our findings do not support the classification of congenital ptosis as a dystrophy.

Key words Levator palpebrae superioris, Histochemistry, Congenital dystrophic ptosis

Congenital dystrophic ptosis can occur as part of a generalised muscular dystrophy but more commonly occurs as an isolated abnormality assumed to be due to a dystrophy of the levator palpebrae superioris (LPS).[1] This histology of LPS has been studied in congenital ptosis.[2–10] Whilst some authors describe dystrophic changes,[2,5,6] others report changes more in keeping with LPS dysgenesis.[3,7,8]

In generalised muscular dystrophies such as Duchenne or facioscapulohumeral dystrophies, there is muscle fibre destruction and regeneration resulting in variability of muscle fibre size and marked hypertrophy of some fibres. There is also extensive endomysial fibrosis. In our study we examined LPS specimens from patients with congenital ptosis for these dystrophic changes and measured the individual muscle fibre diameters.[11] We compared these measurements with diameters from normal LPS specimens taken from live patients. Other studies have used post-mortem specimens for comparison.[8,9]

Histochemical staining has several advantages over paraffin-embedded histological preparations, which are generally formalin fixed. It utilises cryostat sectioning, which maintains normal tissue architecture so that muscle fibre size can be measured and standardised. It identifies the distribution of enzymes in muscle, thus distinguishing different muscle types.[10] LPS muscle fibres can be identified as type 1 (equivalent to mammalian limb slow twitch fibres) and type 2 (equivalent to mammalian limb fast twitch fibres) with this technique.[7,10] We have previously used histochemical staining to examine orbicularis oculi muscle fibre type and proportions in lower lid malpositions and are thus familiar with this technique of examining eyelid specimens.[12] In this study, histochemical staining was used to compare the orthogonal diameters[11] of type 1 and type 2 muscle fibres in patients with normal levator function and congenital 'dystrophic' ptosis.

Materials and methods

Four LPS specimens were obtained from normal lids (3 from normal LPS divided in a bilateral brow suspension procedure and 1 from an exenteration specimen) and 4 LPS specimens were obtained from levator resection procedures for treatment of congenital ptosis. None of the patients had undergone previous lid surgery.

Specimens were stored initially in Croker's medium[13] until they were frozen in liquid nitrogen. Cryostat sections 5 μm thick were cut and mounted directly onto slides. Haematoxylin and eosin, ATPase at pH 9.4 and after preincubation at pH 4.6 and 4.2, and

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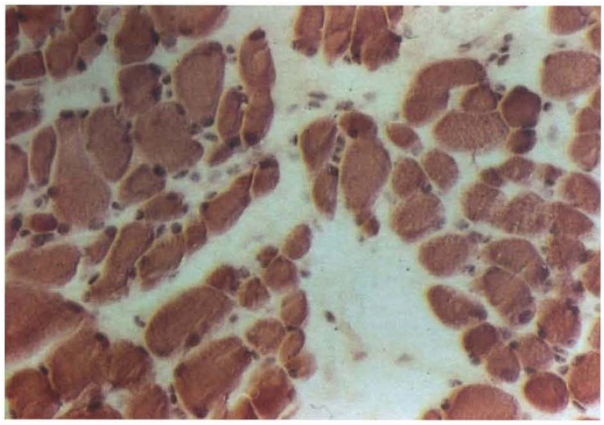

Fig. 1. *Histological section of levator palpebrae superioris stained with haematoxylin and eosin. Magnification ×25.*

NADH-TR stains were used (Figs. 1, 2). The muscle fibre types were most easily identified using ATPase after incubation at pH 4.2, in which type 1 fibres are dark and type 2 fibres are pale (Fig. 2).

The orthogonal diameters of type 1 and type 2 fibres in each specimen were measured using a computer-generated program. In the majority of specimens 100 type 1 and 100 type 2 fibres were measured, although in some specimens (due to artefactual changes, areas of poor staining or small specimen size) fewer were measured, giving a range of 26–100. Magnification was ×40.

The orthogonal diameter is measured by defining the longest diameter of a fibre – the 'major' axis – and measuring the greatest diameter perpendicular to this – the 'minor' axis. This minor axis is considered to be the least likely to be affected by imperfect transverse sectioning.[11] The mean muscle fibre diameters were calculated for each specimen and the two groups (normals and ptosis) compared statistically using the Mann–Whitney *U*-test.

Histograms were constructed to show the frequency and range of muscle fibre diameter in each specimen (Fig. 3).

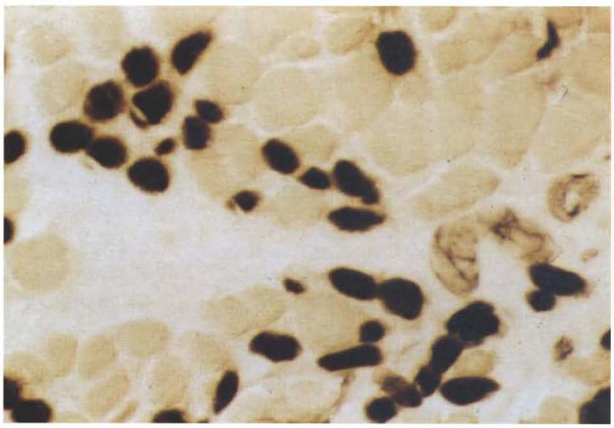

Fig. 2. *Histological section of levator palpebrae superioris stained with ATPase incubated at pH 4.2, showing dark type 1 fibres and pale type 2 fibres. Magnification ×25.*

One author (B.E.) performed the fibre measurements masked to the diagnosis of the specimens. Reproducibility was assessed by measuring five adjacent fibres in three specimens (giving 15 fibres in total) on 10 separate occasions. The 95% confidence limits for reproducibility of muscle fibre measurements were 273.3–278.1 μm.

The specimens were also examined for other dystrophic changes by a neuropathologist (R.O.W.) unaware of the diagnosis of each specimen.

Results

The mean age of patients with normal LPS function was 24.75 years (range 4–79 years) and with congenital ptosis, 4 years (no range). There was no statistically significant difference in the mean diameters of type 1 muscle fibres ($p = 0.685$) or type 2 muscle fibres ($p = 0.486$) between the normal and congenital ptosis groups (Table 1). As muscle fibre size increases towards puberty the data were also analysed excluding the two older patients, but this did not alter the significance of the statistics.

Histograms were constructed to show the distribution of muscle fibre diameters. The type 1 fibre diameter distribution was skewed to the right whilst the type 2 fibre distribution showed a bimodal pattern. Both type 1 and type 2 fibres of patients with congenital ptosis show slight skewing to the right (Fig. 3).

Histological examination of the specimens did not show any evidence of muscle fibre destruction, excessive fibrosis, regeneration, areas of hypertrophy or deposition of extracellular material.

Discussion

The histology of levator muscle has been studied in congenital and acquired ptosis.[2-10] Sutula[2] showed several changes in LPS in congenital ptosis such as loss of cross-striations, random decrease in muscle fibre diameter, sarcolemmal retraction and fibrous and fatty displacement of the muscle fibres. He concluded that the muscle was therefore dystrophic. Berke and Wadsworth[3] showed a decrease in number of muscle fibres in LPS, with the reduction in number being proportional to the severity of the congenital ptosis. Other studies also showed a correlation between the replacement of muscle fibres with fibrous tissue and the degree of ptosis.[5,7] An amorphous extracellular material in some cases of congenital ptosis has also been found.[8] These findings raise the possibility that the changes in congenital ptosis are due to a levator dysgenesis rather than a dystrophy.

Muscular dystrophy is a term used for genetically determined progressive, degenerative myopathies that can show characteristic changes on histochemical staining according to their subgroup.[14] However, congenital dystrophic ptosis generally shows no hereditary component and is non-progressive. The true muscular dystrophies (Duchenne muscular dystrophy, limb-girdle dystrophy, facioscapulohumeral dystrophy,

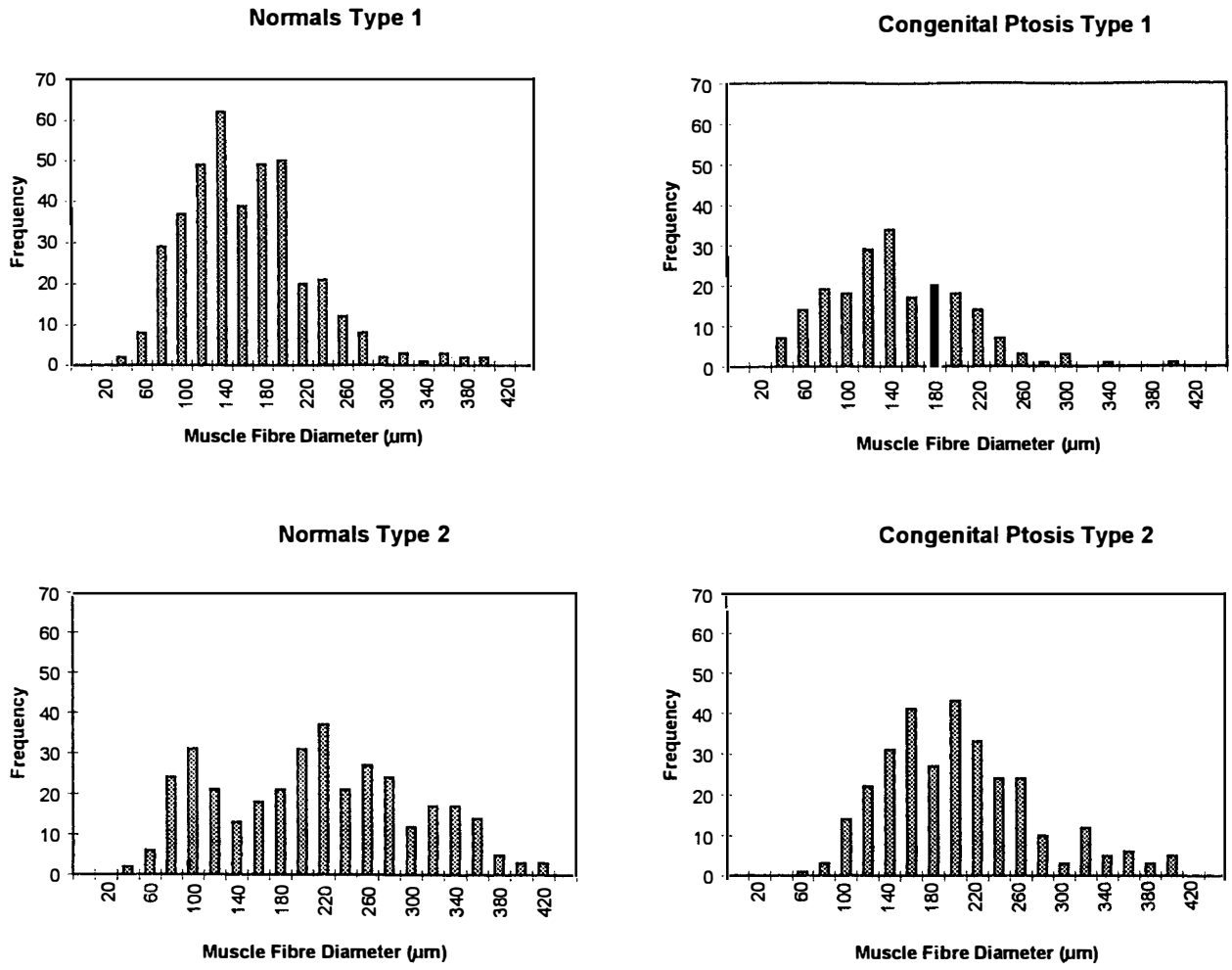

Fig. 3. *The distribution of type 1 and type 2 fibres in normal individuals and patients with congenital ptosis.*

scapuloperoneal dystrophy and oculopharyngeal dystrophy) show evidence of muscle fibre destruction and regeneration. Variability of muscle fibre size and marked hypertrophy of some fibres is typical, as is endomysial fibrosis.

A population of larger muscle fibres would either extend the range of muscle fibre sizes or create a bimodal peak in the distribution pattern of muscle fibre diameters on a histogram. In our study the range of type 2 muscle fibre size was greater in patients with congenital ptosis, but this was caused by a single outlying measurement which, if ignored, left a range similar to that of the normal type 2 fibres. The muscle fibre distributions did not differ markedly between normals and congenital ptosis. However, the small size of our sample could limit the detection of a bimodal distribution where the difference in the two populations of muscle fibre is small.

We have analysed the range, mean and SD of muscle fibre diameters in a group of normal LPS specimens that has only been reported once before on 4 post-mortem LPS specimens.[10] In these 4 specimens the mean diameters of type 1 and type 2 fibres were 18.50 μm and 19.13 μm with a smaller range in fibre diameter than the normals in our study. Our study does not show any statistically significant difference in the size of LPS

Table 1. *Mean orthogonal muscle fibre diameters (μm) and levator function (LF) in LPS specimens from normals and patients with congenital ptosis*

Normals					Congenital ptosis				
Case	Age (years)	Type 1	Type 2	LF	Case	Age (years)	Type 1	Type 2	LF
1	4	15.9	23.3	12	5	4	9.9	21.6	5
2	4	22.9	25.5	15	6	4	21.0	24.2	9
3	12	11.8	12.3	15	7	4	13.9	19.3	4
4	79	19.3	29.0	15	8	4	17.2	21.9	6–7
Mean		17.48	22.53				15.50	21.75	
SD		4.74	7.21				4.76	2.01	

muscle fibres in normals and patients with congenital ptosis. However, the wide range of normal fibre size found and the small number of specimens obtained makes comparison difficult. In our study using histochemical staining of the LPS, we have therefore not found changes in muscle fibre size to support the classification of congenital ptosis as a dystrophy.

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