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Connective tissue changes in surgically overloaded muscle

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Summary. The effects of overload on the connective tissue component of the soleus muscle of the rat have been investigated. Three weeks after tenotomy of its synergistic muscles the soleus underwent considerable increase in weight. This was shown to have resulted from an increase in size of the predominant fibre type. Whilst occasional groups of fibres appeared to have resulted from the splitting of large single fibres, there was no significant increase in the number of fibres in cross-section of the muscle belly. The connective tissue content of the overloaded muscles was investigated using both histological and biochemical techniques. It was found that muscle fibre hypertrophy was accompanied by an increase in the connective tissue component. Furthermore, there was an increase in the proportion of collagen to muscle fibre tissue.

Key words: Surgical overload – Connective tissue – Fibre diameter – Fibre number – Muscle (soleus, rat)

It is known that muscle responds to intensive exercise by synthesizing more contractile proteins (Gordon 1967; Gonyea and Ericson 1976; Goldspink and Ward 1979). Similarly, overloading a muscle by surgical incapacitation of its synergists has been shown to result in hypertrophy of the muscle fibres (Gutmann et al. 1971; James 1976). It would seem likely that when a muscle hypertrophies as a result of excessive use the connective tissue would increase with the increase in muscle mass. In this way the tissue would be able to withstand the large forces developed by the hypertrophied fibres. Although Suomen et al. (1980), using ^{3}H-proline as precursor, have obtained some evidence that collagen metabolism is increased in muscle as a result of exercise, and Turto et al. (1974) have demonstrated an increased activity of prolylhydroxylase during compensatory hypertrophy, there have been very few studies on the collagen content of hypertrophied skeletal muscles. The main purpose of this study was, therefore, to determine whether, when

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a muscle is overloaded, hypertrophy of the muscle fibres is accompanied by an increase in the connective tissue component of the muscle and in particular whether the change is in proportion to the increase in muscle mass.

To ensure that changes in connective tissue following tenotomy of synergists are in fact correlated with hypertrophy it is necessary to determine fibre size and number, since increase in muscle weight alone is not a good indicator of fibre hypertrophy (Vaughan and Goldspink 1979). In this study we therefore have examined histological and biochemical changes in connective tissue in conjunction with changes in the diameter and total number of fibres in the surgically overloaded soleus muscle of the rat.

Materials and methods

Animals

Male rats of the strain CFY and aged 12 weeks were used. The muscle chosen for study was the soleus, a fusiform muscle composed predominantly of Type-I (SO) fibres but in the mature animal containing approximately 20% Type-II (FOG) fibres.

Surgical technique

The animals were anaesthetised with intraperitoneal sodium pentobarbitone and operations were performed on one hind limb. The distal portion of the plantaris and its tendon of insertion were removed and the tendon of insertion of the gastrocnemius was severed just proximal to its junction with the tendon of the soleus. The distal portion of the gastrocnemius was then removed. A sham operation was performed on the contralateral control limb by making a skin incision and separating the muscles. The animals were then left for 3 weeks before being killed and the soleus muscles removed. This period of overload was chosen since a preliminary study showed that considerable hypertrophy occurred in this time.

Analysis of connective tissue

Histology. Muscles were frozen in isopentane, sectioned on a cryostat at 10 μm and stained for connective tissue using Sirius Red (Sweat et al. 1964). This stains the connective tissue of the endomysium, perimysium and epimysium a bright red which, under a green optical filter, appears black and is well contrasted with the pale muscle fibres. The sections were examined under a Leitz microscope to which was attached an image analyser consisting of a video system linked to a small computer. A small area of muscle (0.12 mm^2) was selected, avoiding regions containing dense perimysium and readings taken of the amount of connective tissue. Ten readings were taken for each muscle. The results for experimental and contralateral muscles were compared using a K-S test.

Biochemical assay. Only the middle section of each soleus muscle was used, i.e. that portion which contained no tendon tissue. The samples were weighed, freeze-dried then weighed again. The dry tissue was dissolved in 6NHCl and hydrolyzed by autoclaving. Hydroxyproline was determined using a Technicon autoanalyzer following the method of Grant (1964). Hydroxyproline can be considered to occur exclusively in collagen, thus collagen content can be calculated from the hydroxyproline content of the hydrolysate, (Jackson and Clearey 1967). The analysis was carried out on 24 experimental and 24 contralateral muscles. The results were compared using a paired T-test.

Fibre diameter and number

Six experimental and six contralateral muscles were used. The soleus muscles from experimental and contralateral limbs were weighed then frozen in isopentane, sectioned on a cryostat at 10 μm and stained for myosin ATPase using the method of Tunnel and Hart (1977). Only sections from the mid-belly

region, were used, i.e. those containing all the fibres of the muscle. Sections were examined under a microscope, the eye piece of which contained graduated cross-hairs. The muscle was scanned from the deeper surface to the outer surface a number of times and two estimates made of the diameter of each fibre touched by the centre of the cross-hairs. Measurements were made on 100 SO fibres and approximately 50 FOG fibres (some muscles contained very few FOG fibres). Diameter measurements were then compared using a model-II analysis of variance. Counts were made of the total number of the two main muscle-fibre types using a microprojector. Results were compared using a paired T-test.

Results

Analysis of connective tissue

Histology. Histological examination using Sirius Red indicated that in the overloaded muscles the perimysium was more extensive and that the fibres had a thicker endomysium than in contralateral muscles (Fig. 2). There appeared to be no obvious difference in the thickness of the epimysium. Analysis using the image analyser showed that the amount of connective tissue in a given area of muscle was greater in the experimental than in the contralateral muscles (Table 1).

Biochemical Assay. There was a significant increase in the proportion of collagen in overloaded muscles (Table 1). This increase was found whether the amount of collagen was expressed as per unit wet weight of muscle tissue (8.5 μg collagen in experimental muscles, 7.6 μg collagen in contralateral muscles) or as per-unit dry weight of muscle tissue (38.5 μg in experimental muscles 33,8 μg in contralateral muscles).

Fibre diameter and fibre number. In overloaded muscles SO fibres were found to be of significantly greater diameter (84.1 μm) than in contralateral muscles (72.4 μm) (Table 1). There was no significant change in the diameter of FOG fibres (60.9 μm in experimental and 60.0 μm in contralateral muscles). There was no significant change in the total number of fibres per cross section of muscle, although the percentage of SO fibres increased significantly (Table 1).

Discussion

The model of synergist tenotomy has sometimes been used to study short -term (3–5 days) muscle compensation. During this period weight increases may reflect stretch-induced lengthening of the fibres rather than hypertrophy (Vaughan and Goldspink 1979; Williams and Goldspink 1973) and connective tissue changes may be the result of inflammatory response. Thus studies that have examined the effects of synergist tenotomy on the connective-tissue component of muscle without determining fibre size may not have been looking at the changes accompanying hypertrophy of muscle fibres. An increase in fibre number accompanying hypertrophy has been reported (Hall-Craggs 1970; Sola et al. 1973). The splitting of large fibres into several smaller ones could, by increasing the ratio of endomysium to muscle fibre tissue, increase the proportion of connective tissue within the overloaded muscle. The results described here show that there was no significant increase in fibre number (although there was some apparent fibre splitting in the

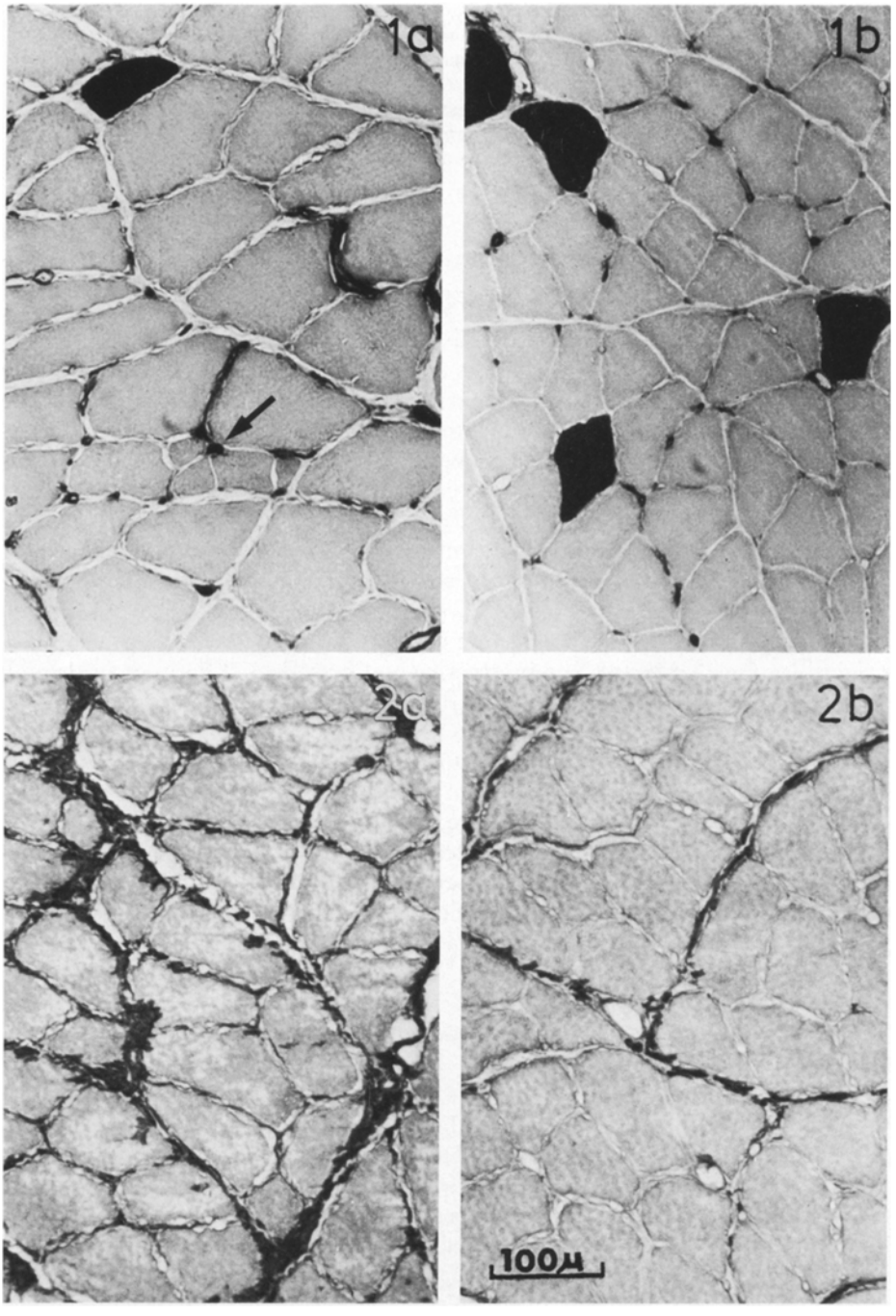

Fig. 1a, b. Transverse section of overloaded (**a**) and contralateral (**b**) soleus muscles stained for myosin-ATPase activity. *Arrow* indicates fibres that may have resulted from the splitting of a single fibre

Fig. 2a, b. Transverse section of overloaded (**a**) and contralateral (**b**) soleus muscles stained for connective tissue (collagen and reticulin) with Sirius Red F3BA

Table 1. Effects of surgical overloading on the connective-tissue component, fibre size, fibre type and fibre number in the m. soleus

	Experimental		Contralateral	
Muscle weight (mg)	275 ± 34		223 ± 13	
Connective tissue concentration (counts per unit area)	796 ± 33*		547 ± 22	
Collagen (μg) per muscle wet wt. (mg)	8.5 ± 0.3*		7.6 ± 0.3	
Collagen (μg) per muscle dry wt. (mg)	38.5 ± 1.52*		33.8 ± 1.54	
	SO	FOG	SO	FOG
Fibre diameter (μm)	84.1 ± 1.2*	60.9 ± 0.8	72.4 ± 1.2	60.0 ± 1.1
SO fibres (%)	92.3 ∓ 0.9*		87.0 ∓ 1.3	
Fibre number	3,037 ∓ 63		3,051 ∓ 52	

* $p < 0.01$

muscle belly (Fig. 1). Thus the increase in muscle weight following the 3-week period of overload was due mainly to a large increase in fibre diameter, the predominant fibre-type (which accounted for over 90% of the total number of fibres) showing a 17% increase in diameter.

The results of the histological study show that hypertrophy of muscle fibres is accompanied not only by an increase in the connective-tissue component of the muscle but also by an increase in the proportion of connective to muscle fibre tissue. This increase in concentration occurred even though in the experimental muscles the areas examined contained fewer larger fibres than the contralateral muscles. This would be expected to reduce the ratio of endomysium to muscle tissue in the overloaded muscles. Thus the increase in connective-tissue concentration is probably greater than is apparent from the results. The results of the biochemical assay support these findings, showing that the percentage of collagen in relation to wet or dry muscle weight is increased in the overloaded muscles. It is not known why this extra connective tissue is produced but it is perhaps required to withstand the strong forces developed by the hypertrophied fibres. It is interesting to note that during stretch-induced hypertrophy in the fowl, total collagen concentration decreased (Laurent et al. 1978): it would appear that hypertrophy induced by different stimuli is accompanied by different connective tissue responses.

The results described here underline the flexibility of muscle tissue and, further, show that this adaptability is not confined to the contractile elements but extends to the non-contractile connective tissue component.

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