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Histochemical Changes in Mouse Soleus Muscle Following Surgically Induced Compensatory Hypertrophy

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ABSTRACT

The size of different types of muscle fiber in the soleus muscles of mice was measured during compensatory hypertrophy. The soleus muscle showed the hypertrophy following the removal of the both the gastrocnemius and plantaris muscle. This hypertrophy was well maintained after 90 days and the cross sectional area of Thpe II fibers of the hypertrophic muscles were enlarged preferentially compared with normal control.

KEY WORDS

Histochemistry, Soleus, Tenotomy, Compensatory hypertrophy

INTRODUCTION

When a skeletal muscle operating in parallel with other muscles is deprived of the cooperative pull of these synergists, it undergoes a remarkable increase in weight. This growth which has been called compensatory hypertrophy (CH), can be experimentally produced by sectioning the tendons of the synergists and has served as a model in many studies on muscle metabolism and growth[1)~4)]. However, most skeletal muscles are composed of heterogeneous populations of muscle fibers that can be distinguished in enzyme histochemical preparations. Some fiber types (Type I) possess a highly oxidative metabolism whilst others (Type II) are predominantly anaerobic[5)]. Although some studies have been carried out on the adaptive responses of muscle fiber types to various types of stress, for example, exercise and functional overload[6)], there is littele data about the long term response to surgically induced CH.

This study was carried out to determine the responses of Type I and Type II fibers in a muscle during CH. The mouse soleus (SOL) muscle was used since it contains equally numerous fibers of both types that can be identified by simple enzyme histochemical technique[6)], This muecle is also capable of maintaining hypertrophic features for prolonged periods following removal of the gastrcnemius muscle and the plantaris muscle[7)].

MATERIALS AND METHODS

Operative techniques. Fourteen male mice (8 weeks old) were randomly selected and kept individually in cage for one week prior to operation. Laboratory Rat Chow and water were available *ad libitum.*

Mid-line longitudinal incision was made posterior to the ankle joint and prolonged proximally over the gastrocnemius muscle under ether anesthesia. The tenotomy operation was included transection of the tendons of entire gastrocnemius and plantaris, with removal of 3-5mm of tissue, so that the muscles could retract well away from SOL. Particular care was taken to ensure that, as far as possible, no nerves were sectioned and blood supply of SOL were preserved. The skin flaps were

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sutured with fine silk and the wound was covered by Nobecutane®.

Histochemical techniques. After 90days of operation, the mice were sacrificed by cervical dislocation. SOL muscles from the operated and contralateral limbs were rapidly exercised and weighed. Thin transverse slices were removed from the mid-belly of each SOL muscle and immediately frozen in isopentane cooled with liquid nitrogen. Tissue was then sectioned at 10 μm in a cryostat at −20℃ and stained to demonstrate myosin ATPase activity. The fiber cross sectional areas and the population of Type I and Type II fibers were obtained with semi-automatic morphometric system (Cosmozone 98, Nikon). Individual cross sectional area of at least 100 contiguous fibers lying in the central region of each muscle section were measured. It has been shown that 100 fibers can be regarded as a sufficiently large sample size for the characterization of most fiber size distributions in skeletal muscle[8]. Following the measurement of each muscle fiber its identity as a Type I or a Type II fiber was established. In the SOL muscle of the mouse the fiber type can readily be identified by its myosin ATPase activity. Type I fibers show moderate staining and Type II fibers are stained heavily for myosin ATPase activity (Fig. 1).

In any study on changes of muscle fiber cross sectional areas particular care must be taken to ensure that comparisons are made at similar sarcomere length. One section from each control and hypertrophic muscle was squashed between slide and cover slip using finger and thumb pressure to re-orientate cut fibers so that their relative sarcomere lengths would be examined by polarized light. No significant difference were found between the sarcomere lengths of control and hypertrophic fibers.

Student's t-test was used to examine the effect of treatment and the histochemical measurement. Significance was accepted at $P < 0.05$.

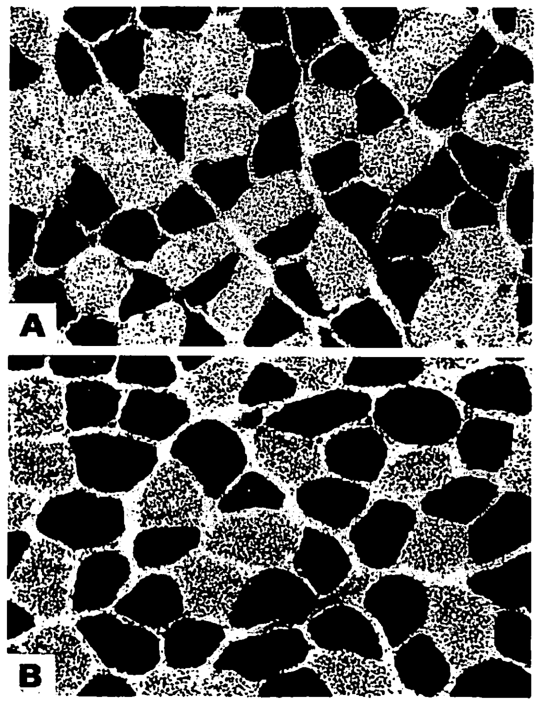

Fig. 1. Transverse sections of normal (A) and hypertrophic (B) soleus muscles stained for ATPase activity. Note the presence of fibers in the field as compared with normal muscle because of the invreased size of Type I and Type II fibers.

Table 1. Cross sectional area and fiber type population in control and hypertrophic muscle

	Type I fiber		Type II fiber	
	Control	Hypertrophic	Control	Hypertrophic
Cross sectional area (μm^2)	941±366	1110±328	931±339	1303±285
Percent increase in mean cross sectional area in hypertrophic muscle (%)	18		41*	
Fiber type population (%)	35±7	39±5	65±7	61±5

Values are mean±SD.

* significant differece ($p < 0.001$) showed that cross sectional area of Type II fibers were larger than that of Type I fibers.

RESULTS

There were increased in the weight of the SOL muscle on surgically tenotomized mice (99±26mg for the control, 127±35mg for the CH) significantly.

The cross sectional area of muscle fibers of the hypertrophic muscles were larger than that of control muscles. The frequency of occurrence of each of the fiber types did not differ following hypertrophy. It was found that the fiber types increased their cross sectional areas by differing amounts (Table 1). Hypertrophic Type II fibers were found to have increased in area (~41%) relatively more than Type I fibers (~18%). Such difference showed to be highly significant by statistical analysis (*P< 0.001).*

DISCUSSION

The results of this present study clearly indicated that Type II fibers increased in size, as measured by their cross sectional area, more the that of Type I fibers during surgically induced CH. In this study the hypertrophy was maintained for a longer period than in most other studies, which have reported either an identical response by the fibers or a prefential increase in size and number of Type I fibers, particularly when the hypertrophy was induced in early life[9]. The results in our study also differed from many previous reports in relation to muscle adaptation following training. and exercise in that the increases in fiber diameter during surgically induced hypertrophy were found to be larger and to affect Type II fibers especially. Some investigators, however, have reported large size increase of up to 40% for Type I fibers and 90% for Type II fibers[10]. The increase in fiber size found in the present study were smaller than those previous reports for CH in the SOL muscle. However, the tissues were prepared for examination using completely different methods, and neither in the previous study nor in the present study have been attempted to make the correction for the shrinkage or swelling of tissue. For example, linear changes in fresh frozen sections can be significantly large and areal changes are correspondingly larger[11].

The results obtained in the present study indicate a differential response of the fiber types. Any study attempting to measure alterations in fiber cross sectional areas following an experimental procedure must take into account the possible effects of an alteration in the number of sarcomeres within the fibers. For example, during CH of Extensor Digitorum Longus muscle additional sarcomeres ould have been added to the ends of the fibers[12]. Consequently, fixing control and experimental fibers at identical lengths, but with more sarcomeres present study in the experimental fibers, would spuriously increase their cross sectional area. The failure to find different sarcomere lengths in Type I and Type II fibers would seem to exclude this possibility. It does not necessarily follow that the prefential increase in the size of Type II fibers is due to any intrinsic properties of the fibers. Hypertrophy would simply be limited by metabolic needs and oxygen supply.

Some investigators have suggested that CH is triggered by mehanical stretching[13], for example, that due to the action of antagonist muscles. Though the precise mechanism of surgically induced CH has still to be elucidated, nevertheless it remains a valuable experimental procedure since the results are reproducible and sufficiently large to be quantitated readily. Moreover contralateral muscles can be used as controls without having to take account of between animal variance. In addition, greater specificity in the choice of hypertrophic muscle can be achieved compared with many exercise regimes, particularly methods involving the running of laboratory cats and the swimming of rodents[14]. The changes found in surgically induced CH may differ fundamentally from those occurring during adaptation to exercise, and this would not be inconsistent with the variations in response to different types human exercise. For example, endurance exercise in long-distance runners produces relatively a littele muscle hypertrophy, but isosmetric training in weight-lifters induced a widespread hypertrophy.

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共同筋腱切断にともなうマウスヒラメ筋の組織化学的変化

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要　　旨

共同筋腱切断によるヒラメ筋の長期的な影響を知るために、14匹のICR系マウスを各7匹ずつ二群に分けた。一群は実験群，他群は対照群とし，前者には麻酔下で腓腹筋と足底筋腱を切断し、ヒラメ筋に肥大を生起させた。90日後，ヒラメ筋を摘出しmyosin ATPase染色を施し，タイプⅠ線維とⅡ線維のいずれがより著しく細胞の肥大が起こるか検索した。その結果，対照群に比べ実験群では筋重量は増加し，タイプⅡ線維の細胞径が選択的に増大していた。