Structural Changes in Human Skeletal Muscle Following Limb-Lengthening by Distraction of the Tibia

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Abstract

Bone distraction is used in orthopedic surgery to correct major limb deformities and limb lengthening discrepancies. Much is known about the response of bone to distraction procedures, but little about responses of the skeletal muscle although the latter may cause important clinical complications.

The object of this study is to determine the effects of distraction on the ability of skeletal muscle to adapt to the extensive limb lengthening.

The present work demonstrates that the mature human skeletal muscle react to bone distraction and adapt to the limb lengthening with an increase in the sarcomere length. Nevertheless the described physiological adaptation was associated to ultrastructural changes in myofibrillar apparatus and to pathological abnormalities in muscle fibers referred to the changes occurring in peripheral nerve fascicles enable to support an important mechanical length increase. Focal endomysial fibrosis with the presence of active fibroblasts and deposition of collagen fibrils was noted in section stained with trichrome stain and in ultrastructural micrographs. These observations are corroborated by determination of collagen content in the biopsed muscles.

Key words: electron microscopy, human muscle, isomyosins, limb lengthening, structural changes.

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The ability of mature muscle fibers of mammals to adapt to experimental lengthening by adding serial sarcomeres is well established [15, 16]. It is not known whether human muscle fibers can adapt to increases in length so important as imposed by bone distraction surgery. Bone distraction is used in orthopedic surgery to correct major limb deformities and limb lengthening discrepancies. Recently this method has been successfully applied to limb-lengthening of dwarf-achondroplastic subjects. The procedure involves osteotomy followed by progressive distraction of the two parts of bone using an external frame [5, 8, 3, 2]. Much is known about the response of bone to distraction procedures, but little about responses of the skeletal muscle although the latter may cause important clinical complications.

The object of this study is to determine the effects of distraction on the ability of skeletal muscle to adapt to the extensive limb lengthening. Morphological and ultrastructural analyses were performed on skeletal muscle biopsies of three young achondroplastic patients. The biopsies were taken before surgery and in two patients 4 months after surgery, when an increase of 10 cm in the tibia length has been achieved. In a third patient the second biopsy was 24 months after surgery to study the long-term adaptation of skeletal muscle to limb lengthening. The analyses of fibre types and diameter and of the sarcomere number and length, were performed by an automatic interactive imaging analyzer system. The electrophoretic analysis of actin and myosin heavy chains, the measurement of total protein content and of collagen content were also performed on the biopsies of one patient.

Patients and Methods

Three young achondroplastic patients (14, 16, 24 years old), were selected for limb lengthening by tibial callus distraction using a modified Wagner technique [2]. Trans-

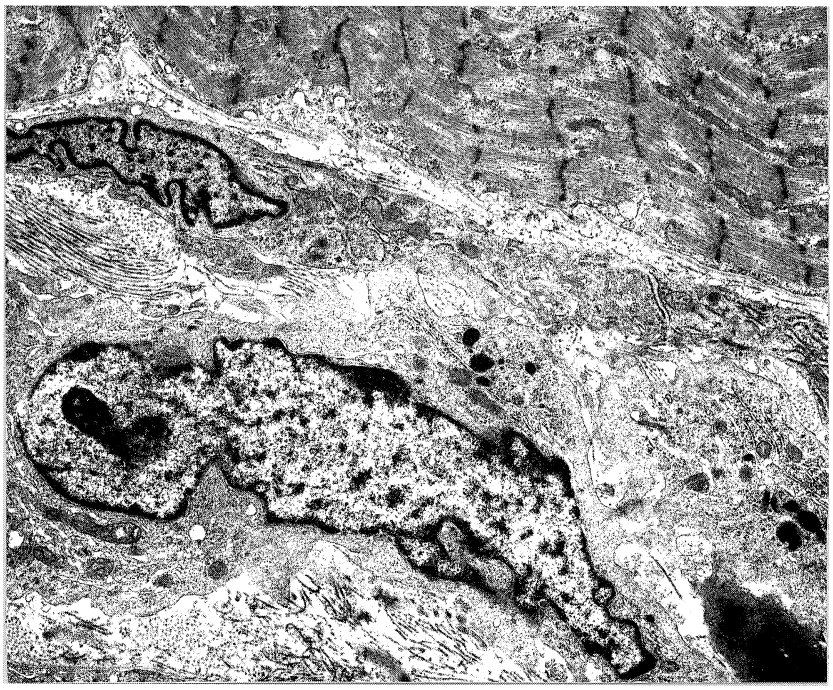

Figure 1. Electron micrograph of an atrophic fiber: in the endomisium a fibroblast showing a well developed RER, is surrounded by dense bundles of collagen fibers (x 4500).

verse osteotomy was given to the middle of the tibia. The bone was lengthened by 1mm daily (four distractions of 0.25 mm every 6 hours) for 4 months from day 5 after surgery, to produce a 48% (10 cm) limb length. The first muscle biopsy was collected from the tibial anterior muscle at the time of surgery. In two case the second biopsy was collected 4 months after surgery by the percutaneous needle technique. In one case the second biopsy was collected two years post-operatively. Small pieces of the muscle sample were fixed in Karnovsky's fluid and processed for electron microscopy. Longitudinal sections were mounted on copper grids, contrasted with uranyl

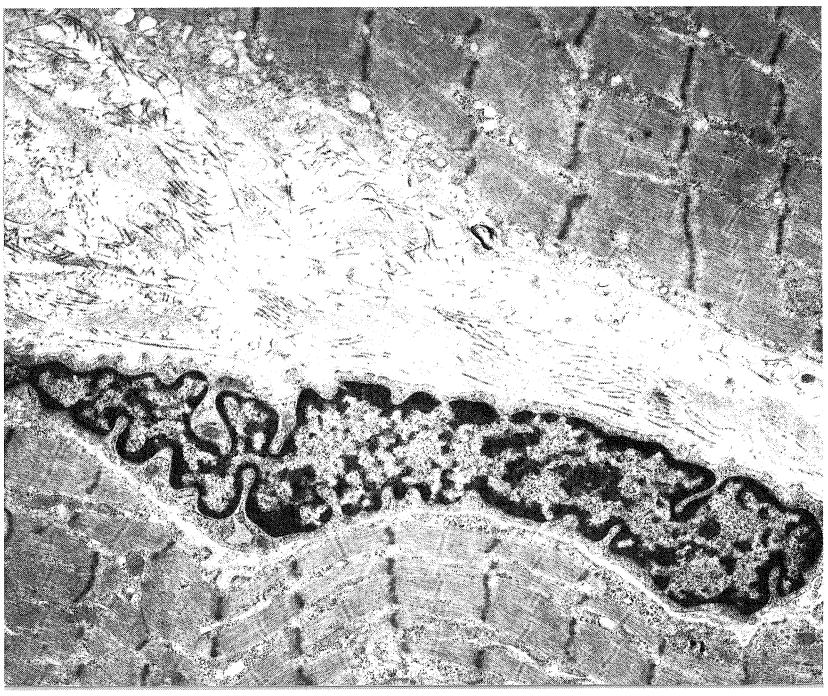

Figure 2. Ultrastructural detail of a satellite cell closely apposed to a skeletal muscle fiber (x 4500).

acetate and lead citrate and examined in a Zeiss EM 109 electron microscope. The number and length of sarcomeres were measured per 10 μm length of the central region of ten fibers per each sample. The major portion of each biopsy was oriented in tragacanth gum, frozen in isopentane cooled by liquid nitrogen and stored at -70°C. The frozen muscles were serially cryosectioned (10-20 μm sections) for histological, histochemical and electrophoretic analyses. The fiber type composition was determined by myofibrillar adenosine triphosphatase analysis, using pre-incubation pH values of 4.3, 4.6, and 9.6. The fiber types were identified according to [1]. The quantitative analysis of the diameter and percentage of the fiber types was performed using an interactive automatic image analyzer (IBAS I and II system). Total protein content was measured by Lowry method [7], collagen content was measured according to [17] modified by [4]. SDS-PAGE according to [6] was used to separate myosin heavy chains (MHC) and actin from a few cryostat sections (20 μm thickness) of the biopsies [11]. Densitometric scanning of the 7.5%-12.5% polyacrylamide slabs allowed us to measure the percent content of the different myosin heavy chains and the MHC/Actin ratio [11].

Results and Discussion

Table 1 presents morphological and histochemical characteristics of the tibialis anterior muscle before and after the distraction of the tibia. Four months after muscle lengthening the percentage of type 1 fibers is increased and there is a slight increase in the diameter of both type 1 and of type 2 fibers; some scattered angulated, atrophic fibers are present. Two years after surgery the percentage of type 1 fibers is increased in comparison to the situation at the time of the surgery, but the fiber diameter is smaller than at time of the surgery. The mosaic pattern of fiber type becomes disturbed, with the occurrence of large clusters of type 1 fibers, sometime involving whole fascicles. These patterns, known as type grouping are markers of denervation reinnervation events. Proliferation of the endomysial connective tissue is evident, particularly 2 years after surgery.

The electron microscopic analysis shows loss of sarcomere register and streaming of the Z disc in some lengthened fibers (Fig. 1). Very damaged atrophic fibers are rare. In these fibers myofibrils are unrecognizable and scattered myofilaments fill the whole fiber. Some damaged fibers are characterized by central nuclei. On the other hand satellite cells are quiescent (fig. 2) and immunocytochemical analysis demonstrated rare embryonic myosin-positive myofibers (results not shown). Scarce and small mitochondria are present inside the fibers and some satellite cells are often apposed to the fiber sarcolemma. The loose connective tissue proliferate between the fibers and is characterized by the presence of numerous fibroblasts interspersed with dense bundles of collagen fibers leaning the muscle fibers (Fig. 2). The morphometric analysis of sarcomere length and number per area unit before and after surgery is reported in table 2. After limb lengthening the mean number of sarcomeres was reduced, since their length was increased.

In table 3, the percent of myosin heavy chains (MHC), the total protein and collagen content, the MHC/actin ratio are reported. In the patient studied the percent of Type 1 MHC agrees very well before and after surgery with the fiber type composition obtained by histochemical ATPase. Compare data in table 1 and 3. The MHC/Actin ratio is in the normal range before and after surgery, suggesting a normal trophic state of the muscle, at least in the biopsed material. On the other hand, after surgery the percent content of collagen increased eight time, in agreement with the morphological data. Total protein content is normal after surgery, while the decreased content in the sample before surgery reflects the sampling of adipose tissue in the bioptic material as suggested by the protein/collagen ratio which is normal.

The present study has been performed to show the possible skeletal muscle abnormalities after surgical limb-lengthening procedures, it was also designed to provide informations on the capacity of human mature muscle

Table 1. Fiber type composition and fiber diameter.

	Case 1 4 months after surgery			Case 2 4 months after surgery			Case 3 24 months after surgery		
	Type1 Diameter μm	Type2 Diameter μm	Type 1 %	Type1 Diameter μm	Type2 Diameter μm	Type %	Type1 Diameter μm	Type2 Diameter μm	Type1 %
Before surgery	44 ± 2.6	52 ± 3.0	56	48 ± 2.6	54 ± 3.2	48	40 ± 2.8	54 ± 3.0	46
After surgery	46 ± 2.4	56 ± 3.4	70	48 ± 3.6	58 ± 2.4	56	36 ± 2.2	50 ± 2.4	80

Values are mean ± SD

Table 2. Number and length of sarcomeres.

	Case 1 4 months after surgery		Case 2 4 months after surgery		Case 3 24 months after surgery	
	Number/100 μm^2	length μm	Number/100 μm^2	length μm	Number/100 μm^2	length μm
Before surgery	34.62 ± 2.20	2.92 ± 0.50	36.20 ± 2.30	3.10 ± 0.60	38.30 ± 2.80	2.80 ± 0.80
After surgery	24.80 ± 2.30	4.62 ± 0.96	27.22 ± 2.40	4.8 ± 0.80	26.60 ± 0.7	4.60 ± 0.78

Values are mean ± SD

Table 3. Total protein and collagen content, myosin heavy chain and actin in tibialis anterior of case 1, before and 4 months after surgery

	MHC type			MHC/Actin	Protein	Collagen	Prot/Coll %
	2A	2B	1				
Before surgery	47	-	53	2.45	10.8	0.5	21.6
After surgery	17	7	76	2.10	22.0	4.0	5.5

fibers to adapt to a change in functional length. Recently limb lengthening by callus distraction in rabbit was widely performed, but there have been few reports concerning the modifications of skeletal muscle in this condition. Some authors showed hypertrophy of type 1 slow fibers which play an important role in the tonus of the muscle, and slight hypotrophy of type 2 fast fibers [9]. The older surgical procedure of human limb-lengthening following the method given by Ilizarov have been performed to correct major limb deformities and limb lengthening discrepancies, but they were frequently accompanied by muscle weakness and contractures [12]. More recently the surgical procedure has been modified and now the method of limb-lengthening by callus distraction has been utilized also for elongation of the lower extremities of dwarf achondroplastic subjects, as described in the present report. Limb elongation ranging from 8 to 10 cm was generally satisfactory for the achondroplastics which obtained beneficial physical and psychological improvements. Nevertheless in some patients there is muscle contracture and neuromuscular complications which limit the extent and rate of lengthening achieved. In patients after 10 cm of limb lengthening four months after surgery there are minor modifications of fiber diameters. The histochemical analysis reveals a type 1 fiber predominance with type grouping indicating muscle reinnervation. In the patient biopsed 24 months after surgery, type 1 fibers are almost doubled, the diameter of both fast and slow fibers being slightly reduced. These abnormalities are indicative of changes in peripheral nerve system which have been also demonstrated after electrophysiological investigations performed on our patients and described also in a previous study [10]. Clinical observations of numerous patients operated with the original Ilizarov method have demonstrated muscle weakness in the involved limb after tibial lengthening. In an electrophysiological study on patients after limb lengthening by bone distraction, abnormalities in nerve conduction of deep and superficial peroneal nerves were described [18]. The alterations are in agreement with those seen in experimental studies following calf limb lengthening. These studies performed on vascular, nervous and muscular components of limb showed abnormalities in peripheral nerve fascicles consisting in wallerian degeneration of large myelinated nerve fibers at the major rate of bone distraction [19].

Experimental investigations on rabbits in which the functional length of the skeletal muscle is altered by immobilisation in a lengthened position or by postural malalignment, demonstrated that the muscle fibers adapt by adding serial sarcomeres [13, 14]. Recently the ability of the mature rabbit muscle to adapt to surgical bone distraction has been investigated. At the lower rates the increase in length could be accounted by an increase in serial sarcomeres and the muscle fibers showed no atrophy

or damage, thus indicating a good adaptation to the imposed length. At the higher rates of distraction, fewer sarcomeres were added to the muscle fibers, the sarcomere length was significantly increased, and the muscles presented evidence of damage or atrophy [16]. In the present report electron microscopic analysis of muscle fibers regarded mainly the myofibrillar apparatus that is primary involved in the muscle lengthening. After 10 cm lengthening, all achondroplastics showed a significant increase in sarcomere length if compared with measures performed before the surgery. All muscle showed also evidence of structural damage, loss of register of sarcomeres and Z disc streaming or smearing. In the present post-operative conditions the human muscle fibers adapt to the limb lengthening certainly by increase in sarcomere length while we have not information on eventual production of serial sarcomeres or formation of new material to maintain optimum sarcomere length along the muscle fibers. The present work demonstrates that the mature human skeletal muscle react to bone distraction and adapt to the limb lengthening with an increase in the sarcomere length. Nevertheless the described physiological adaptation was associated to ultrastructural changes in myofibrillar apparatus and to pathological abnormalities in muscle fibers referred to the changes occurring in peripheral nerve fascicles enable to support an important mechanical length increase. Focal endomysial fibrosis with the presence of active fibroblasts and deposition of collagen fibrils was noted in section stained with trichrome stain and in ultrastructural micrographs. These observations are corroborated by determination of collagen content in the biopsed muscles. Changes in the interstitial connective tissue components leading to increase muscle stiffness and to the loss of joint movement were also described in the rabbit tibialis anterior muscle following distraction of the tibia [16]. Work are in progress to study if the described muscle abnormalities seen after 10 cm bone distraction are reversible.

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