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EFFECTS OF MANDIBULAR ADVANCEMENT ON THE HISTOCHEMICAL CHARACTERISTICS OF THE LATERAL PTERYGOID MUSCLE IN YOUNG RATS

by

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Abstract

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The objective of this experimental study was to investigate the response of the lateral pterygoid muscle to changes in its functional length during mandibular advancement induced by functional appliance treatment. The experiment was carried out on 24 young female Sprague-Dawley rats, initially 20 days old. Appliances causing a 3mm advancement of the mandible were designed, constructed and bonded to the mandibular incisor teeth of the experimental rats. The sample was equally divided into four main groups: two control and two experimental. In one experimental group, the appliance for mandibular advancement was kept in place for a period of ten days. The second experimental group wore the appliance for twenty days. The control groups, one for each experimental period, were left untouched. At the end of the ten and twenty day periods, the left and right lateral pterygoid muscles were removed and immediately

frozen in isopentane chilled with liquid nitrogen. Serial cross-sections, 8 μm thick, of the fresh- frozen biopsies were processed for histochemical demonstration of alkali and acid stable myosin ATPase activity.

Interpretation of the data was based on intra- and inter-group comparisons of (1) muscle fiber types, (2) fiber diameter, and (3) number of muscle fibers. With the application of Student's t-tests, few significant differences were detected between control and experimental groups. Histochemical examination of muscles from treated and untreated animals demonstrated similar patterns of differentiation into Type I, Type II A and Type II B fibers. Type I fibers, mainly with aerobic or oxidative profiles. They come in use at low forces and are relatively fatigue resistant. Type II A fibers with both aerobic and anaerobic capabilities. They are able to maintain repetitive phasic contractions and are fatigue resistant. Type II B fibers, mainly with an anaerobic or glycolitic profile. These have shorter contraction time and show rapid fatigue. Type II A fibers predominated in control as well as experimental groups both at ten and twenty days. The differences between the number of this muscle fiber types and the two others were statistically significant ($p < 0.01$). It was also found that the type II A fibers decreased in number with increasing age (i.e., from 10 to 20 days). This was also statistically significant for both control ($p < 0.05$) and

experimental ($p < 0.01$) groups. Statistical analysis showed a significant ($p < 0.05$) decrease in diameter of type II B fibers in the experimental group at 10 days when compared to the control group. Comparisons of fiber diameters within groups demonstrated type II A fibers to be significantly larger than other fiber types in both experimental and control groups at 20 days ($p < 0.05$) and the experimental at 10 days ($p < 0.01$). The total number of fibers showed no significant differences between groups.

Based on the variables investigated in this study, adaptive responses of skeletal muscle to altered function appeared to be only transitory (type II B fibers were significantly reduced in diameter in treated animals at 10 days). When the lateral pterygoid muscle functions in a shortened position induced by anterior displacement of the mandible, changes occur in the muscle before skeletal responses. Due to continuous growth during treatment, the distance between the condylar and pterygoid process will increase and thus the muscle must continuously adjust. It is concluded that similarities in the lateral pterygoid muscle in treated and untreated animals is due to skeletal growth responses that occurred with and without treatment. Further, this study allowed comparisons with other investigations on normal postnatal developmental patterns of skeletal muscle.

DEDICATED

TO

My father Wulfran, who has walked with me, side by side throughout my entire career; who with his unlimited love and trust has guided my life and highly contributed to the fulfillment of my goals. May God bless him forever.

My mother Eva, my brothers and sisters for being there when I most needed their love and support.

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INTRODUCTION

In considering any orthodontic therapy it is important to have a thorough understanding of the various tissue responses during active treatment. Changes occur which involve both hard and soft tissues.

With functional orthodontic appliances in the treatment of class II maloclussions the mandible is displaced downward and forward in a teeth-apart position. Besides effects on the dentoalveolar process which are generally acknowledged to take place, it is believed by many that such anterior mandibular displacement stimulates condylar growth resulting in permanent increase of mandibular size. Browne (1959) and Marschner and Harris (1966), in clinical studies that considered total mandibular length increments in addition to mandibular positioning, noted a significantly higher rate of mandibular growth in a group of patients treated with functional appliances than in an untreated control group. In experimental studies, irrespective of whether non-human primates or rats have been used, anterior mandibular displacement has been shown to produce visible changes at the head of the condyle (Charlier and Petrovic, 1969; Petrovic A. et al., 1975). Thus, both clinical and experimental investigators have described in the literature bone alterations in response to functional orthodontic treatment.

However, adaptive changes induced by altered function are not limited to skeletal responses. Changes may also occur within related musculature. In a study of altered functional position of the lower jaw in growing monkeys McNamara (1972, 1973), showed that the

superior head of the lateral pterygoid muscle plays an active role in determining the anteroposterior position in the mandible. Contractions in the superior head of the muscle may position and stabilize the disc anteriorly simultaneously as they protract the condyle along the articular eminence.

The importance of clarifying the relationship between muscle and bone during orthodontic treatment has been recognized by many investigators (Petrovic and Stutzman, 1972; McNamara, 1972). An additional question arises as to the extent to which surrounding musculature is involved in the long-term stability of such treatment. Moss (1962, 1968, 1970) postulated the "functional matrix concept" in which the functional matrices play a primary role in the control of form and position of skeletal parts.

Numerous studies have been published which analyze responses within muscles to functional alterations. Muscles may adapt to alterations in functional length by changes in the number of sarcomeres in series, fiber size and in the distribution of fiber types. Tabary et al., 1972 examined the effect of immobilization at different lengths on the number of sarcomeres in series along the muscle fibers. As a result of this experiment, the number of sarcomeres in series decreased when the muscle was immobilized in the shortened position, while when immobilized in the lengthened position, the number of sarcomeres increased. The same phenomena occurred in studies done by Oudet and Petrovic (1978), in which the

number of sarcomeres in series decreased in the lateral pterygoid muscle as a result of mandibular deviation.

Several investigators have provided descriptive data on the histochemical characteristics of muscle fibers within the muscles of mastication (Hiraiwa, 1978; Ringqvist, 1978; Maeda et al., 1981; Eriksson and Thornell, 1983). However, this approach has not been used extensively to describe responses within these muscles to functional changes.

Thus, the present study is designed to analyze the effects of mandibular advancement on the histochemical characteristics of the lateral pterygoid muscle. It will provide information related to muscle adaptation in response to induced shortening by prolonged anterior displacement of the mandible.

LITERATURE REVIEW

Effects of Muscle on Skeleton

It is known that functioning muscles exert in a general way, significant morphogenetic effects on skeletal tissues to which they are attached. They may influence size as well as shape of bony structures. Historically, many attempts have been made to more precisely define the role of the neuromusculature in postnatal osseous morphogenesis. Tower (1937) reduced muscle function by immobilizing the hind legs of growing animals. The long bones were found to be deficient in thickness, and the areas of muscle attachment were poorly developed. Scott (1954, 1961) reported that if muscular paralysis occurred during the active growth period, the bones of the involved limb tended to become reduced in size.

Regarding muscle function and its role on facial form, many theories have been put forward. Brodie (1941) has stated that facial skeletal patterns are genetically predetermined and environmental influences are only secondary. However, according to Van der Klaauw (1945) the craniofacial complex comprises at least thirty relatively independent functioning units, each governed by its own determinant. Moss (1962) further developed Van der Klaauw's ideas into the "functional matrix" concept which states that the adaptability of skeletal structure to functional patterns and extrinsic environmental conditions is of primary importance. Partly based on these concepts, an interrelationship of muscle function and bone

growth has been realized through many experimental studies (e.g., Petrovic and Stutzmann, 1972). However, the exact nature of this association is still under investigation.

Muscle Growth, Muscle Function and Length

With regards to muscles, many experiments have been carried out to determine the site of longitudinal growth. Earlier it was believed that interstitial growth occurred throughout the muscle fiber (Haines, 1932; Alder et al., 1960), while now it is held that sarcomeres are added serially at the ends of the fibers (Mackay and Harrop, 1969; Goldspink, 1974; Williams and Goldspink, 1976).

The functional component in the regulation of muscle growth is not completely known. However, it has been clearly demonstrated that function is significantly involved, since in paralyzed muscles, fiber growth is decreased (Comer, 1956; Ahmed, 1966). It appears that the length of the muscle belly is related to the distance of contraction (Crawford, 1954).

On the other hand, passive muscle stretching without active contraction is sufficient to cause muscle fiber growth (Crawford, 1972; Williams and Goldspink, 1976).

Experiments designed to study the effect of muscle immobilization in a lengthened position show that sarcomeres are being added (Tabary et al., 1972; Williams and Goldspink, 1973). Conversely, if a muscle is immobilized in a shortened position, they showed that sarcomeres are lost. However, little is known regarding muscle response when the muscle is allowed to function with simultaneous

shortening, which is a condition produced by some functional jaw orthopedic appliances. When the mandible is advanced as a therapeutic measure in a skeletal class II maloclussion, the distance between the two attachments of the lateral pterygoid muscle is reduced while the muscle is still allowed to function.

One factor which appears to influence the activity of the condylar cartilage is the lateral pterygoid muscle. Based on the reduction observed in the number of cells undergoing mitosis, Petrovic and Stutzmann (1972) concluded that cutting the lateral pterygoid muscle reduces the growth of the condylar cartilage. Experimental data are also available indicating that this cartilage responds with increased growth to an orthopedic appliance which increases the activity of the lateral pterygoid muscle (Charlier et al., 1969; McNamara, 1972).

Studies with postural hyperpropulsors allowing progressive mandibular advancement (Petrovic, 1978) have shown increase in lateral pterygoid muscle activity, recorded electromyographically, which subsequently brought about an increase in the rate and amount of condylar cartilage growth. The results of this experiment are explained in the light of a cybernetic model in which much has yet to be elucidated.

Since it has been shown that the lateral pterygoid muscle plays an important role in condylar growth, it would be of significance to examine if changes within the muscle other than sarcomere number, occur during treatment with a functional orthopedic appliance.

Identifying such changes would be of value in assessing prognosis of treatment effects.

Parameters of Developmental Muscle Change

Information is available on muscle fiber-type changes in response to alterations in functional circumstances. Tabary (1972) and Williams and Goldspink (1973) studied the effect of immobilization, and Goldspink and Ward (1978) analyzed changes in rodent muscle fiber types during postnatal growth, under-nutrition and exercise.

Histochemical studies have been done to describe fiber-type profiles in human masticatory muscles (Ringqvist, 1978; Eriksson et al., 1981). It has been established that the fiber types are closely related to the functional demands of the muscles. The fiber type composition in the adult lateral pterygoid muscle indicates a capacity for endurance during continuous work at relatively low forces which accords with the stabilizing effect on the mandibular joint attributed to this muscle (Eriksson et al., 1981).

Following alteration in the functional length of masticatory muscles of female rhesus monkeys, Maxwell and coworkers (1981) studied histochemical properties, muscle fiber cross-sectional area, muscle fiber length and oxidative capacity. An intraoral appliance was used to displace the mandible infero-posteriorly which caused a 15% lengthening of masseter and temporalis muscles. They found that stretching these muscles within physiological limits did not significantly alter the proportion of fiber types. However, it did appear

to cause a reduction in the oxidative capacity of type I and type II A fibers. They also found that surgical detachment of the masseter muscle with surgical reattachment resulted in a significant decrease in the mean cross-sectional area of type I fibers in the anterior masseter muscle. The authors concluded that neither surgical manipulation of the masseter alone nor the appliance were sufficient to induce significant response. However, the combination of the two stimuli resulted in marked effects.

Further studies dealing with the adaptation of orofacial soft tissues in response to orthopedic appliances in orthodontic treatment are still necessary for a better understanding of their function including tissue effects.

STATEMENT OF THESIS

The response and subsequent adaptation of the orofacial musculature to dentofacial orthopedics is an area which has attracted much investigative attention. The main reason for this is that the theoretical aim for so called functional orthodontic treatment is to influence neuromuscular behavior. It is however, debatable to what extent such adaptational alterations can be achieved. More basic data are needed to evaluate tissue effects, short-term as well as long-term, of such therapeutic procedures.

Thus, the objective of this research project is to test the hypothesis that adaptational alterations will occur in the lateral pterygoid muscle when shortened by prolonged anterior displacement of the mandible.

An acrylic appliance was bonded to the anterior mandibular teeth of an experimental group of young rats. The amount of induced mandibular advancement was 3 mm. Another group of the matched animals served as controls, and was left untouched. Two different experimental periods were used: (1) one in which the appliance was left in place for 10 days and (2) another in which it remained in place for 20 days. At the end of each period the right and left lateral pterygoid muscles were removed for histochemical and morphometric analysis.

Data were obtained regarding (1) muscle fiber types, (2) fiber diameters, and (3) number of fibers in muscle fiber types.

MATERIALS AND METHODS

Sample

A total of 24 female Sprague-Dawley rats[1] was used to carry out the experiment. They were 20 days old at the beginning of the experimental period and their weights ranged from 34.0 to 49.2 grams. To accelerate independency from mother, the pups were force weaned at 18 days post-partum without causing any weight loss.

All animals were kept in standard laboratory housing under similar conditions: (1) Room temperature 65-75°F; (2) Humidity 55-60%, (3) Light cycle of 14 hours per day. The animals were fed Enfamil (infant formula) and softened regular rat food[2]. Body weights were recorded daily throughout the experiment.

The rat is an experimental animal that can withstand both the physical and psychological stresses involved in this experiment. In addition, there are data available in the literature with regard to normal muscle fiber growth and histochemical profiles of the different musculature for this species.

Lateral Pterygoid Muscle

The lateral pterygoid muscle originates from the sagittal part of the palatine bone and also from the pterygoid bone. It inserts on the condyloid process. The muscle seems to be active in all mandibular positions and movements except retrusion (Molin, 1973; Lehr and Owens, 1980).

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[1]Zivic Miller Laboratories Inc., Allison Park, PA

[2]Purina Rat Chow, Ralston Purina Co., St. Louis, MO

Grouping and Experimental Period

The sample was equally divided into four main groups (Table 1): (1) Experimental 1 (appliance for mandibular advancement for a period of 10 days, at the end of which lateral pterygoid muscle was removed for muscle histochemistry), (2) Experimental 2 (appliance for mandibular advancement for 20 days followed by histochemical analysis), (3) Control 1 (10 days), and (3) Control 2 (20 days).

TABLE I

GROUPS AND EXPERIMENTAL PERIODS FOR HISTOCHEMISTRY STUDY

EXPERIMENTAL TIME / GROUP	10 Days	20 Days
EXPERIMENTAL	6 animals for mandibular advancement	6 animals for mandibular advancement
CONTROL	6 animals left untouched	6 animals left untouched

Design of Appliance for Mandibular Advancement

An acrylic appliance was designed on the mandibular incisors of a dry rat skull of comparable size to the experimental animals. It permits feeding while maintaining functional mandibular protrusion (Figs. 1, 2A &B).

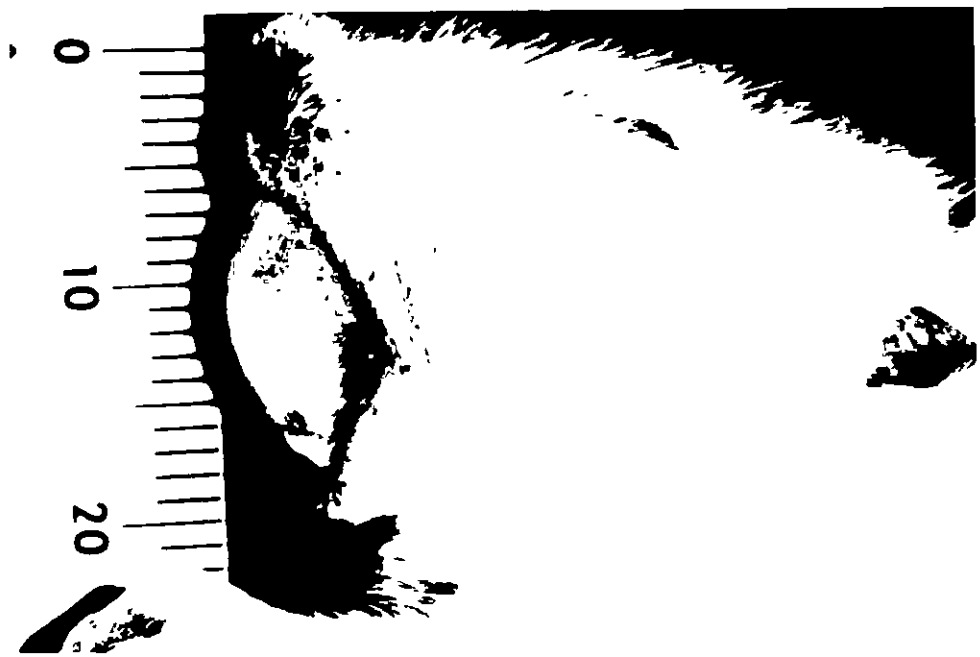
The appliance was bonded with orthodontic resin[3] to the anterior mandibular teeth of the experimental animals under general anesthe-

[3]Concise, Dental Products/3M, St. Paul, MN

Figure 1: Appliance for mandibular advancement. The appliance was made of acrylic and designed on a rat skull of comparable side to the animals used in the experiment.



Figure 2 (A and B): Appliance bonded to the mandibular incisor teeth. It maintains mandibular protrusion and permits feeding.


0
10
20


0

sia. The mandible was displaced anteriorly 3 mm. Lateral skull radiographs were taken before and after appliance placement to indicate the induced displacement (Fig. 3).

The animals were force-fed for approximately two days following appliance placement to prevent excessive weight loss.

Anesthesia

For both the bonding procedures and the removal of the lateral pterygoid muscles, each animal was anesthesized with an intramuscular injection of Ketamine HCl[4], at a dose of 100 mg/kg body weight. With this dose, the anesthetic period lasted approximately 2 hours.

Procedure for Surgical Removal of Lateral Pterygoid Muscle

The animal to be dissected was randomly selected from its group. Each animal was anesthesized with an intramuscular injection of ketamine HCl. All instruments for the dissection procedures were placed on the surgical table in advance: scissors, scalpel, surgical blade #15, hemostats, tissue forceps and discoid.

The facial hair was shaved off to clean the surgical area. The skin was freed extending over the pre-auricular area, anteriorly to the nasal and oral regions, superiorly to the temporal regions and inferiorly to the neck area. The masseter, buccinator, zygomaticus and temporalis muscles were deflected to expose the ramus of the mandible, the temporomandibular joint and the zigomatic arch. The latter was removed to better visualize the mandibular condyle (Fig. 4).

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[4]Ketaset, Veterinary Products, Bristol Laboratories, Syracuse, NY

Figure 3: Radiograph of experimental rat immediately following appliance placement. The amount of anterior displacement was 3 mm.

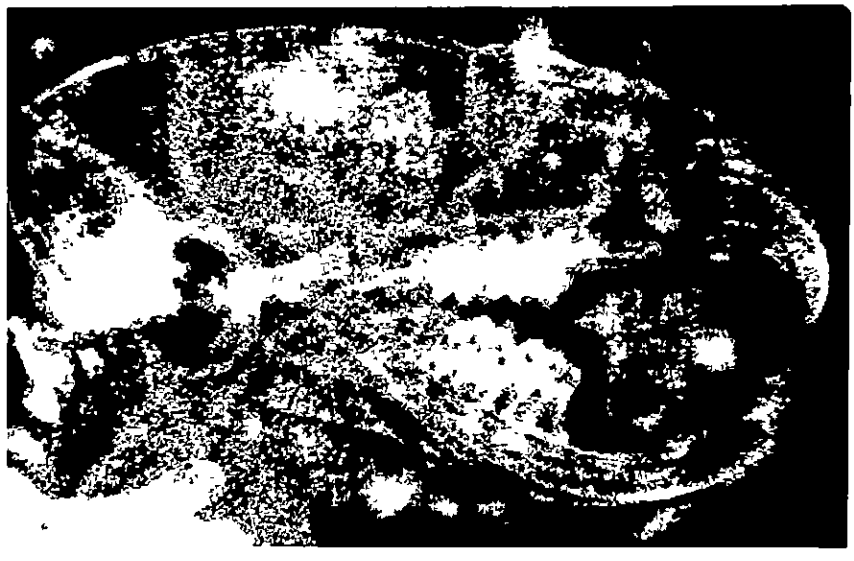


Figure 4: View of left ramus and mandibular condyle after deflection of masseter buccinator, zigomaticus and temporal muscles during dissection.

Figure 5: View of left lateral pterygoid muscle. It originates on the pterygoid plate and inserts at the condyle.





At this point, the pterygoid muscle could be seen, including its insertion on the medial surface of the mandibular condyle. With a pair of scissors a cut was done at the neck of the condyle. The mandible was moved inferiorly to fully expose the lateral pterygoid muscle (Fig. 5). By pulling gently at the condyle the muscle in its total length could be appreciated. It was carefully detached at both of its attachments. Small blocks of tissue were excised from the middle part of the muscle, and immediately frozen in liquid nitrogen for histochemical procedures.

Both right and left muscles were removed in all rats. The duration of the surgical procedure was 45-60 mins for each animal. Following muscle removal, the animals were killed with an overdose of sodium pentobarbital[5] by cardiac injection.

Muscle Histochemistry

The procedures described in this section were performed to facilitate the examination of possible changes in muscle fiber types, as well as in size and number of fibers. An analysis of such changes will allow us to compare the variables mentioned between the lateral pterygoid muscle in the induced shortened position and the normal controls.

Muscle Biopsies

The middle part of right and left lateral pterygoid muscles from all experimental and control animals were prepared for

---

[5]Diabutal™, Diamond Laboratories, Inc., Des Moines, IA

histochemical examination. The gastrocnemius and soleus muscles were also biopsied and used as reference material since their normal histochemical profiles are known (Armstrong and Phelps, 1984).

The following steps were employed for the preparation of muscle tissues prior to histochemical processing (Dubowitz and Brooke, 1973).

1. The middle portion of the lateral pterygoid muscle was removed under general anesthesia. Biopsies from the soleus or gastrocnemius muscles were also taken.
2. The muscle specimen was placed in Cryomold[6], which was half way filled with Cryoform[7]. After orientation of the specimen more cryoform was added to completely cover the tissue.
3. The cryomold with specimen was immediately frozen by plunging it into isopentane, cooled to -160°C with liquid nitrogen. The tissue block was wrapped in aluminum foil and stored in a freezer at -85°C.
4. Serial cross-sections, 8 µm thick, were cut using a cryostat[8].
5. The sections were positioned on warm, clean, chrome-allum treated slides, air-dried at room temperature for an hour prior to histochemical processing.

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[6]Tissue-Tek II, Lab Tek Div., Miles Laboratory, Inc., Naperville, IL.

[7]Tissue-Tek II. Lab Tek Div., Miles Laboratory, Inc., Naperville, IL.

[8]International Cryostat, IEC, Model CTO, Needham Heights., MA.

Methods for Calcium-Activated ATPase

The mechanism of the ATPase reaction is as follows (Sheehan and Hrapchak, 1980): Sections are incubated in a solution containing adenosine triphosphate (ATP) substrate and calcium. ATPase present in the skeletal muscle tissue hydrolyses the substrate, and the products formed are adenosine diphosphate (ADP) and orthophosphate. The released phosphate combines with the calcium present to form colorless calcium phosphate. When the tissue is exposed to the cobalt chloride solution, the cobalt is exchanged for the calcium and a colorless precipitate of cobaltous phosphate is now present at the sites of enzymatic activity. Upon treatment with ammonium sulfide, a black insoluble precipitate of cobaltous sulfide is formed and permits visualization of the products of enzymatic activity.

The procedure involves the following steps (Chayen et al., 1969):

1. Pre-incubation at either pH 4.3, 4.6 or 10.3 for five minutes. Reaction medium consists of:
   (1) 0.2 M sodium acetate (27.2 g/1000 ml), 45 ml
   (2) 0.2 M acetic acid (11.55 ml gs/1000 ml), 55 ml
   The pH is adjusted with 0.1 M HCl and 0.2 M NaOH.
2. Blotting of the pre-incubation solution remaining on the slides with clean tissue paper.
3. Incubation at pH 9.4. All pre-incubated sections are incubated in a freshly prepared reaction medium at 37°C

for 30 minutes. Reaction medium consists of:

(1) 0.1 M sodium barbiturate solution (2.06 g/100 ml) 71 ml

(2) ATP, 76 mg

(3) 2.4 dinitrophenol, 30 mg

(4) 2% calcium-chloride solution (10.5 g $CaCl_2$ $2H_2O$/100 ml), 5 ml

(5) Distilled water, 35 ml

4. Wash in three changes of 1% calcium chloride for 2 minutes in each bath.
5. Rinse in running distilled water for 2 minutes.
6. Transfer to 1% cobalt chloride solution for 2 minutes. Wash in three changes of the cobalt chloride solution for 2 minutes.
7. Wash thoroughly in running distilled water for 4 minutes to remove unabsorbed cobalt.
8. Place in dilute ammonium sulfide (0.5% v/v) for 1 minute.
9. Wash in distilled water for 2 minutes.
10. Mount the sections with A-Q[8] mounting medium.

ATPase activity is shown by a brown-black precipitate of cobalt sulfide. Table II shows a summary of histochemical profile according to myosin ATPase activity.

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[8]Hopkins and Williams, Chadwell Health, Essex, England

TABLE II

SUMMARY OF HISTOCHEMICAL PROFILE
ACCORDING TO MYOSIN ATPase ACTIVITY

	Type I	Type II A	Type II B	Type II C
pH 9.4	light	dark	dark	dark
pH 10.3	light	dark	dark	dark
pH 4.6	dark	light	dark	dark
pH 4.3	dark	light	light	grey

Morphometric Procedure

All slides were examined under low magnification (x25), to discard slides with damaged and/or improperly stained sections. Of the remaining slides, one slide per animal was selected for (1) fiber diameter measurements and (2) fiber type count.

On a white paper a rectangle of 14 x 15 cms was drawn and the outlines of the muscle fibers that fell within this area were traced under low magnification (x25) using a Leitz light microscope with a drawing tube. When a fiber touched the borderline of the area, its entire outline was completed, even though part of the fiber may have extended beyond the outline area.

One area from each lateral pterygoid muscle was randomly selected and traced. The number of muscle fibers in each retangle was counted. There were approximately 200 fibers per sample. The diameters for each of these fibers were measured by taking the maximum distance across the lesser aspect of the muscle fiber (Brooke, 1973). This procedure was used to avoid artifacts introduced by oblique sectioning of the muscle which will give an artifically large measurement. Measurements for fiber diameters were taken with a Helios precision needle-jaw dial caliper.

After completion of each tracing, identification of the different fiber types was performed under the same microscope. Three types of muscle fibers were found in all the specimens examined, each of which was marked with a different color to make the distinction: (1) Blue, was used to identify Type I Fibers, (2) Red,

for the Type II A, and (3) Green, for the Type II B. Fiber type counts were done at pH 4.6 where the three muscle fiber types were distinguishable.

Analysis of Data

All 24 animals included in the control and experimental groups were weighed daily. The average weight gain for each animal was calculated by subtracting the initial weight (weight recorded on the first day of experimental period) from the final weight (weight recorded on the last day of experimental period), and divided by the experimental period. The sum of the average weight gains in each group was divided by the number of animals in the group (6 animals for each control and experimental groups). Statistical analyses by means of Student's t-test were performed to compare weight gains between the groups. Daily average group weight gains were also plotted in graph forms.

After having identified the different fiber types, the number of fibers were counted from each animal in randomly selected fields. The sum of the number of fibers within each muscle fiber type was divided by the number of animals in the group. Histograms were used to graphically illustrate differences between the groups. Intra- and inter-group comparisons were made through Student's t-tests to evaluate statistical significance.

The same analyses were done for determination of muscle fiber diameters.

Histograms were included in this study to show differences between the means and their standard deviations because they permit rapid and easy evaluation of differences of the variables involved although they do not reveal statistical significance.

Intra- and inter-group comparisons were performed for all control and experimental groups by means of Student's t-distributions. This type of statistical approach is a simple way to compare group means for statistical significance.

RESULTS

The Results are presented in Tables II through X and Figures 6, through 15.

Weight Gain

Body weights in both experimental groups (10 and 20 days) decreased during the first two days after the appliance for mandibular advancement was bonded in place (Fig. 6). It was observed that the animals were able to adapt to the appliance and did not seem to experience any significant problems in feeding behavior. In spite of this observation, the appliance must have interfered with normal intake of food. When the average group weight gains of the experimental animals for both periods was compared to that of the controls, significant ($p < 0.01$) less weight gain was apparent for the experimental groups (Table III).

Muscle Histochemistry

Muscles are composed of fibers which are different in their histochemical, biochemical and physiological characteristics. Based on these, muscle fiber types can be classified into:

1. Type I fibers, mainly with aerobic or oxidative profiles. They come in use at low forces, and are relatively fatigue resistant.

Figure 6: Average weight gain for experimental and control groups (10 and 20 days). The weight gain of each animal within groups was recorded daily and the values were averaged.

_____ Control (10 days)

___.___.___ Experimental (10 days)

__ __ __ __ Control (20 days)

__..__..___ Experimental (20 days)

AVERAGE GROUP WEIGHT GAIN

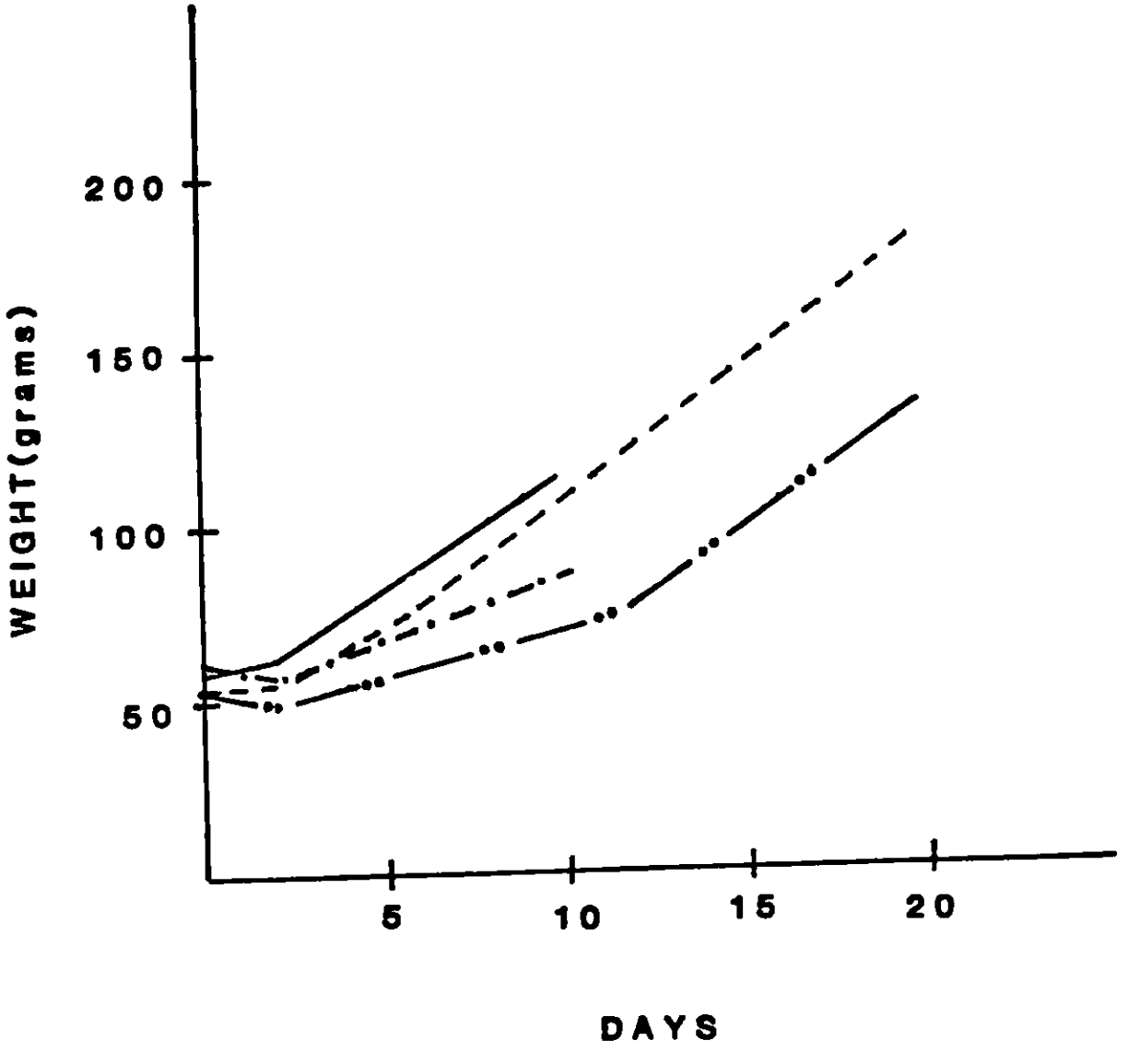


TABLE III

AVERAGE GROUP WEIGHT GAIN

EXPERIMENTAL AND CONTROL GROUPS

(10 And 20 Days)

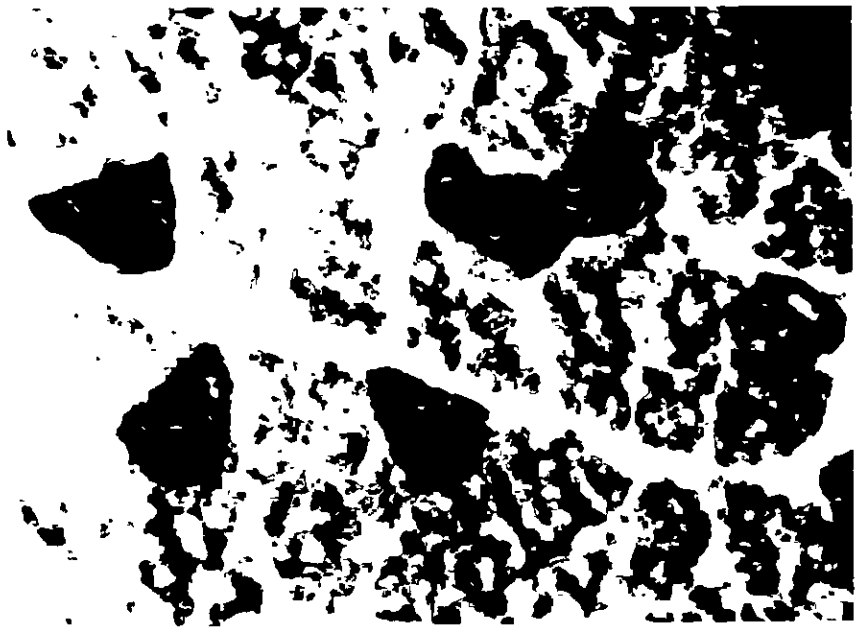
CONTROL (10D)		EXPERIMENTAL (10D)		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
5.48	0.71	2.4	1.38	4.86	10	$p<0.01$
CONTROL (20D)		EXPERIMENTAL (20D)		Test $U_1 = U_2$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
6.76	0.39	3.93	1.48	4.53	10	$p<0.01$

α = Level of Significance

Figure 7: Cross-sections of normal rat soleus muscle.

A. Routine ATPase at pH 9.4 x 495

B. Modified ATPase preincubated at pH 4.6 x 495



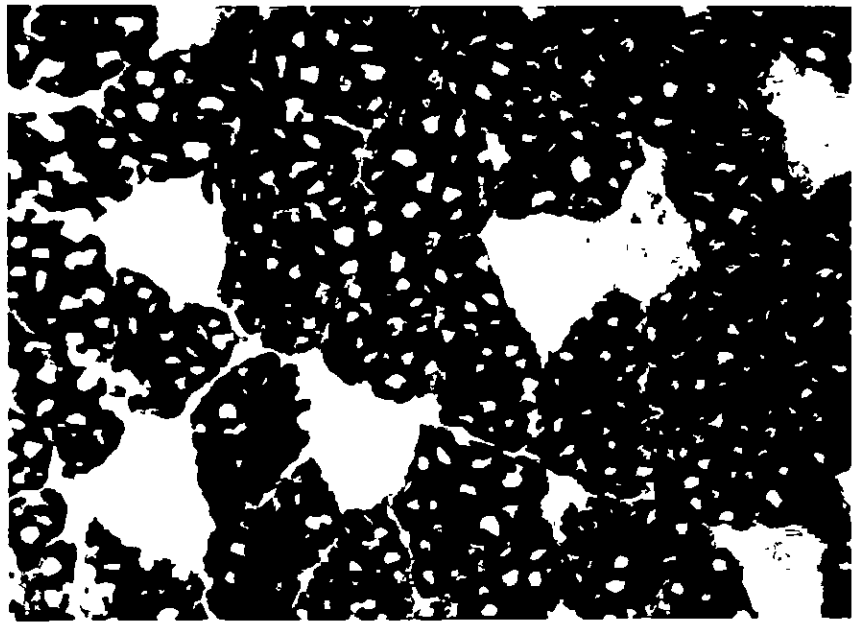
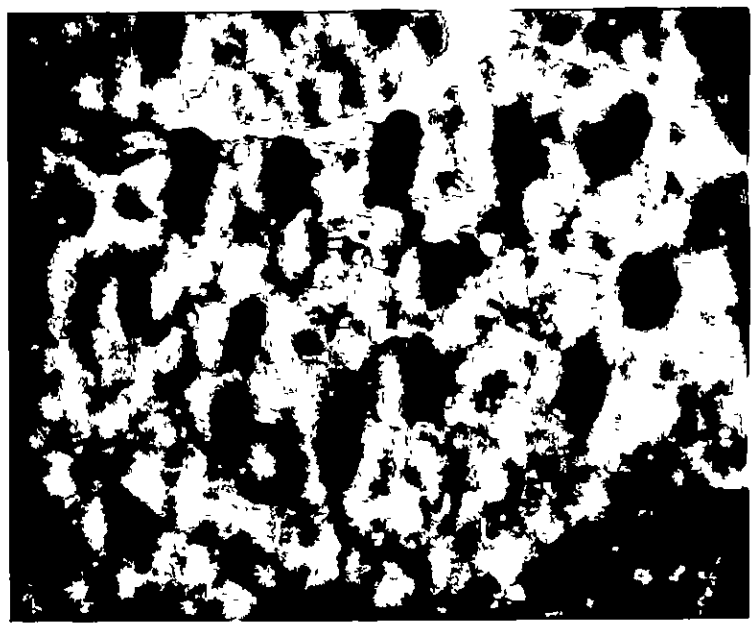


Figure 8: Muscle histochemistry of a control lateral pterygoid muscle at 10 days (ATPase at pH 4.6) x198.

Figure 9: Muscle histochemistry of an experimental lateral pterygoid muscle at 10 days (modified ATPase preincubated at pH 4.6) x198.



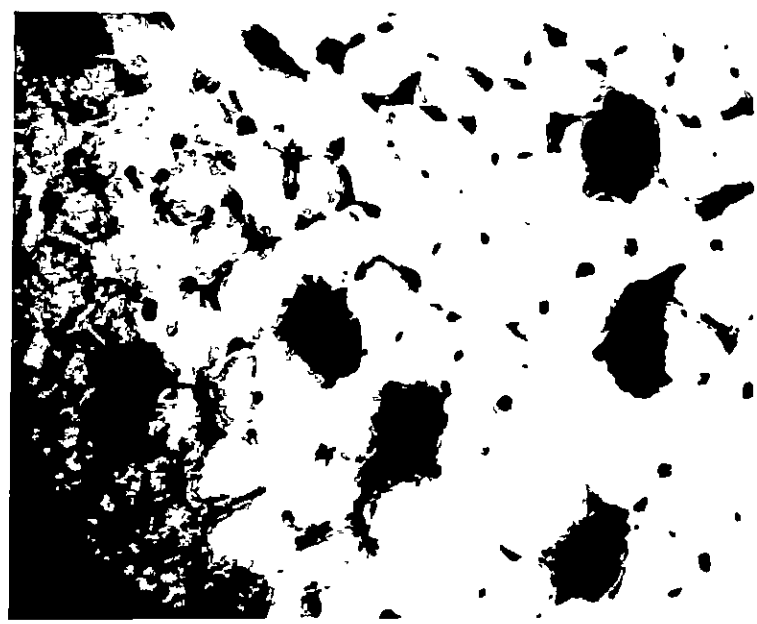
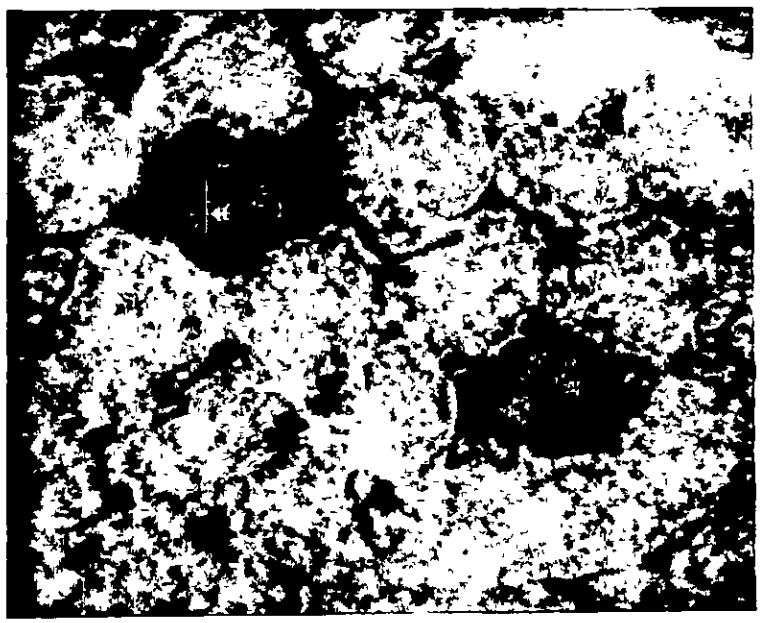


Figure 10: Muscle histochemistry of a control lateral pterygoid muscle at 20 days (ATPase at pH 4.6) x495.

Figure 11: Muscle histochemistry of an experimental lateral pterygoid muscle at 20 days (modified ATPase preincubated at pH 4.6) x 495.





means for the number of fibers in these muscle fiber types are shown in Figures 12 and 13. A comparison between control and experimental groups at 10 and 20 days showed no significant difference in the number of fibers within each type (Table IV). However, type II A was the predominant fiber in all groups. It had significantly ($p < 0.01$) higher values both at 10 and 20 days (Table V, VI).

Intergroup comparisons were also done to evaluate possible differences in the number of fibers within each type at 10 and 20 days for both control and experimental groups. The results are presented in Table VII. Both control and experimental groups at 20 days revealed a significant ($p < 0.01$, and $p < 0.05$, respectively) decrease in type II A fibers when compared to those at 10 days.

Morphometric Data

Intra- and inter-group comparisons of muscle fiber diameters were done to evaluate the influence of anterior mandibular displacement on cross-sectional dimensions of the lateral pterygoid muscle. Mean diameters of muscle fibers within each fiber type for control and experimental groups are shown in Figures 14 and 15. Statistical analysis by means of a Student's t-test showed a significant decrease ($p < 0.05$) in diameter of type II B fibers in the experimental group at 10 days when compared to the control group. Type I and type II A fibers did not show significant differences probably due to the large variance (Table VIII). No significant differences between the control animals and those with mandibular advancement were found for the experimental period at 20 days. Tables IX and X

show results for comparisons of fiber diameter at 10 and 20 days within control and experimental groups, respectively. Type II A fibers were significantly ($p < 0.05$ and $p < 0.01$) larger than type I fibers in both groups at 20 days and in the experimental at 10 days. That the control group at 10 days did not show such differences may be due to the large variance.

Total Number of Muscle Fibers

Student's t-tests were also used to compare the total number of fibers between control and experimental groups. Results in Table X show no significant difference between the groups for either of the experimental periods. It can also be seen in this table, that the number of fibers in the experimental groups (10 and 20 days) is greater (although not statistically different) than in the controls.

Figure 12: Mean number of fibers within fiber types control and experimental groups (10 days). Error bars indicate standard deviation.

MEAN

NUMBER OF FIBERS IN FIBER TYPES

10 DAYS

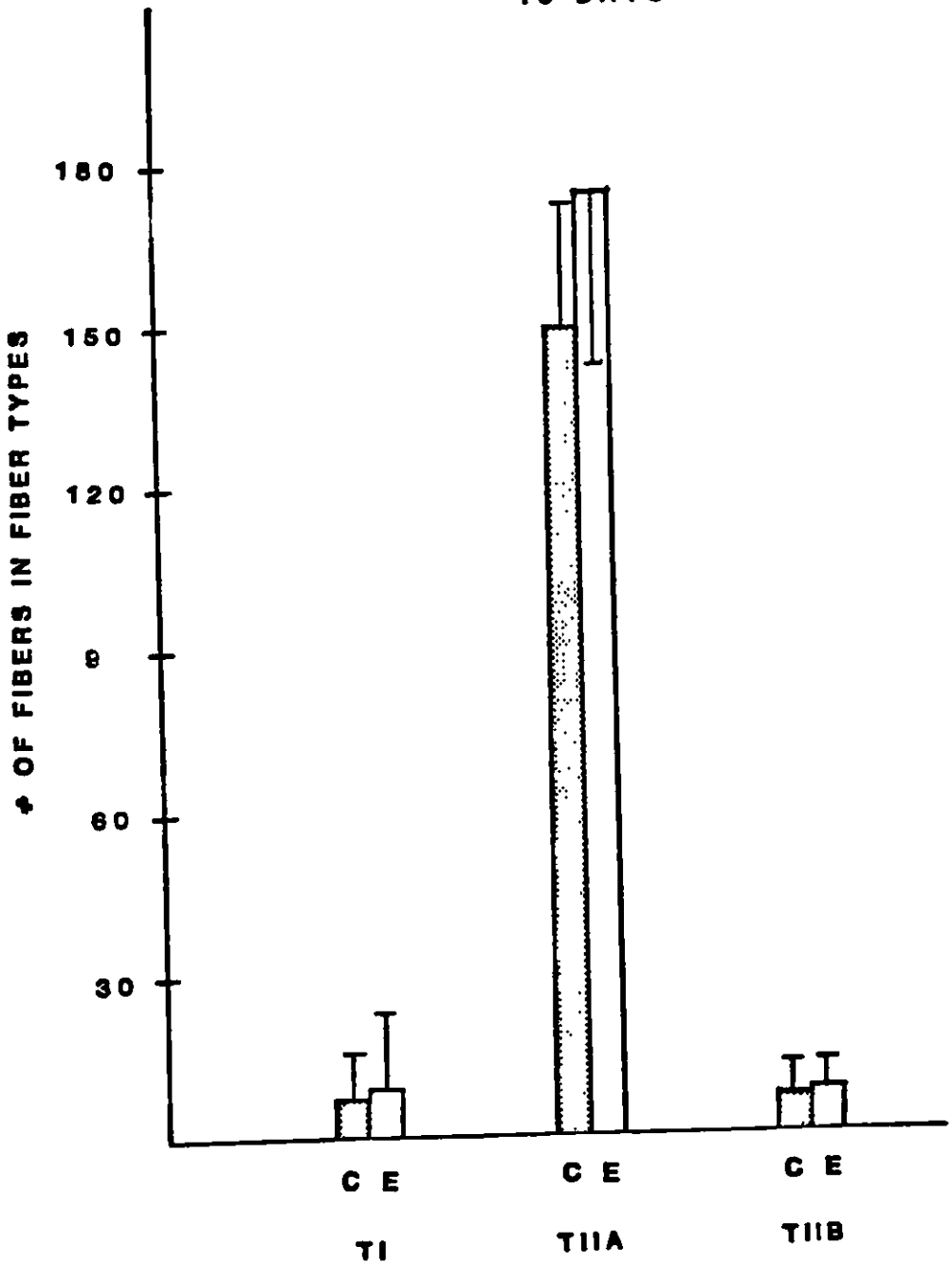


Figure 13: Mean number of fibers within fiber types control an experimental groups (20 days). Error bars indicate standard deviation.

MEAN

NUMBER OF FIBERS IN FIBER TYPES

20 DAYS

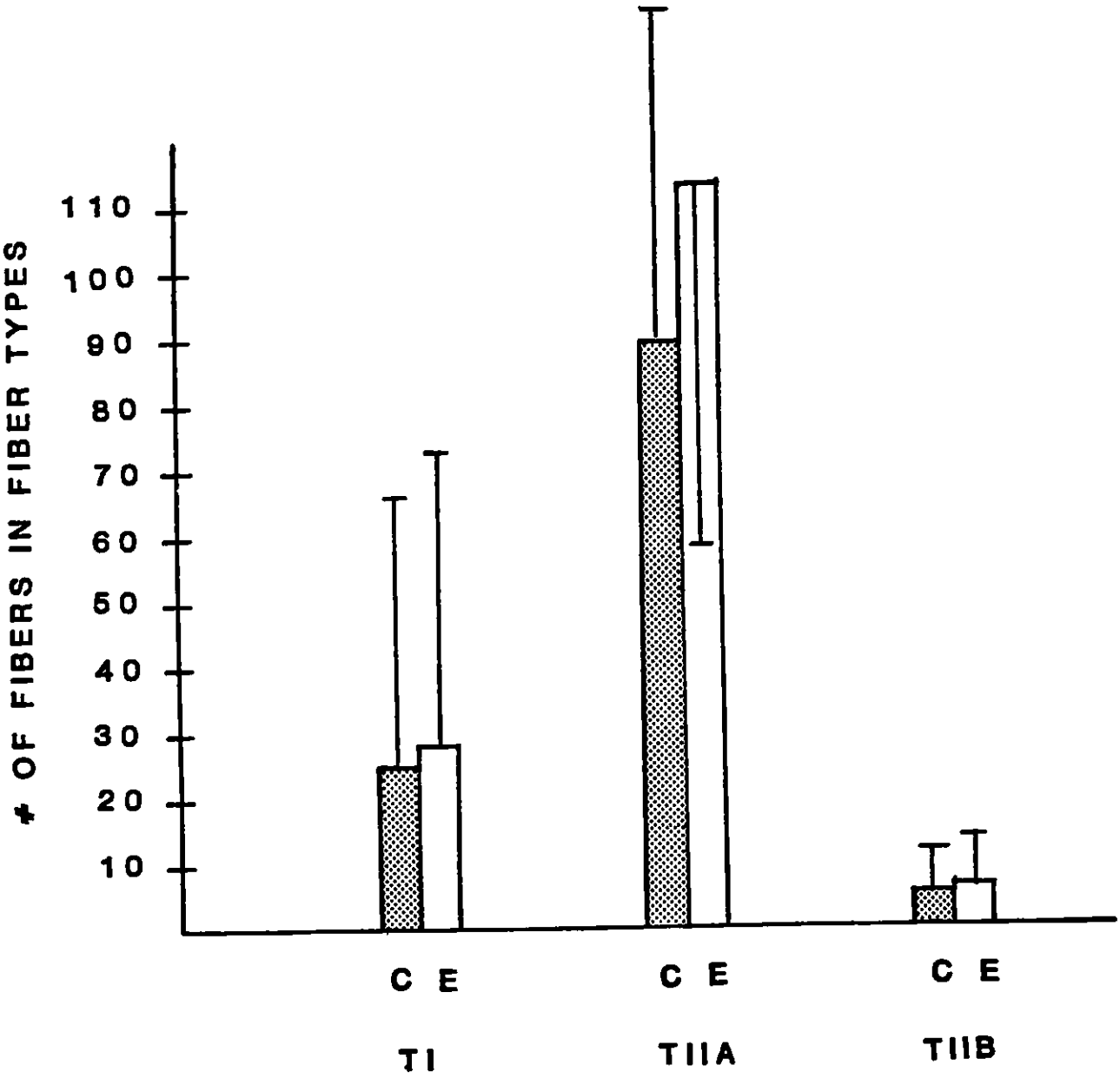


TABLE IV

NUMBER OF FIBERS WITHIN MUSCLE FIBER TYPES
CONTROL AND EXPERIMENTAL GROUPS
(10 AND 20 DAYS)

10 DAYS

Variable	CONTROL		EXPERIMENTAL		TEST $U_1 = U_2$		
	$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
Type I	8.6	5.89	9.16	14.23	0.09	10	NS
Type II A	143.16	28.06	174.3	31.99	1.79	10	NS
Type II B	7.8	5.08	8.3	7.6	0.05	10	NS

20 DAYS

Variable	CONTROL		EXPERIMENTAL		TEST $U_3 = U_4$		
	$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
Type I	25	41.88	29.50	45.55	0.18	10	NS
Type II A	89.16	40.97	108.6	54.98	0.72	10	NS
Type II B	4.83	7.55	7.5	8.35	0.60	10	NS

α = Level of Significance

TABLE V

NUMBER OF FIBERS WITHIN FIBER TYPES
CONTROL GROUPS (10 AND 20 DAYS)

10 DAYS

Type I		Type II A		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
8.6	5.89	143	28.60	11.28	10	$p < 0.01$

Type I		Type II A		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
8.6	5.89	7.8	5.08	0.25	10	NS

Type IIA		Type II B		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
143	28.6	7.8	5.08	11.40	10	$p < 0.01$

20 DAYS

Type I		Type II A		Test $U_3 = U_4$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
25	41.88	89.12	40.97	3.80	20.	$p<0.01$

Type I		Type II B		Test $U_3 = U_4$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
24	41.88	4.83	7.55	5.51	10	$p<0.01$

Type IIA		Type II B		Test $U_3 = U_4$		
$\bar{X}_1$	SD1	$\bar{X}_2$	SD_2	t	df	α
89.16	40.97	4.83	7.55	4.96	10	$p<0.01$

α = Level of Significance

TABLE VI

NUMBER OF FIBERS WITHIN FIBER TYPES
EXPERIMENTAL GROUPS (10 AND 20 DAYS)

10 DAYS

Type I		Type II A		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
9.16	14.23	174.3	31.99	11.55	10	$p < 0.01$

Type I		Type II B		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
9.16	14.23	8.3	7.6	0.13	10	NS

Type II A		Type II B		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
174.3	31.99	8.3	7.6	12.36	10	$p < 0.01$

20 DAYS

Type I		Type II A		Test $U_3 = U_4$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
29.50	45.70	108.5	51.70	2.80	10	$p < 0.05$

Type I		Type II B		Test $U_3 = U_4$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
29.50	45.70	7.5	7.94	1.16	10	NS

Type II A		Type II B		Test $U_3 = U_4$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
108.5	51.70	7.5	7.94	4.73	10	$p < 0.01$

α = Level of Significance

TABLE VII

NUMBER OF FIBERS BETWEEN FIBER TYPES
CONTROL GROUPS (10 AND 20 DAYS)

10 DAYS		20 DAYS				
Type I		Type I		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
8.6	5.89	25	41.88	0.95	10	NS
Type II A		Type II A		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
143	28.60	89.16	40.97	2.63	10	$p<0.05$
Type II B		Type II B		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
7.8	5.08	4.83	7.55	0.08	10	NS

NUMBER OF FIBERS BETWEEN FIBER TYPES
EXPERIMENTAL GROUPS (10 AND 20 DAYS)

10 DAYS		20 DAYS				
Type I		Type I		Test $U_1 = U_2$		
$\bar{X}_2$	SD_2	$\bar{X}_4$	SD_4	t	df	α
9.16	14.23	29.50	45.70	1.04	10	NS
Type II A		Type II A		Test $U_1 = U_2$		
$\bar{X}_2$	SD_2	$\bar{X}_4$	SD_4	t	df	α
174.3	31.99	108.5	51.70	2.65	10	$p<0.05$
Type II B		Type II B		Test $U_1 = U_2$		
$\bar{X}_2$	SD_2	$\bar{X}_4$	SD_4	t	df	α
8.3	7.6	7.5	7.94	0.18	10	NS

α = Level of Significance

Figure 14: Mean fiber diameters: control and experimental groups (10 days). Error bars indicate standard deviation.

MEAN FIBER DIAMETER

10 DAYS

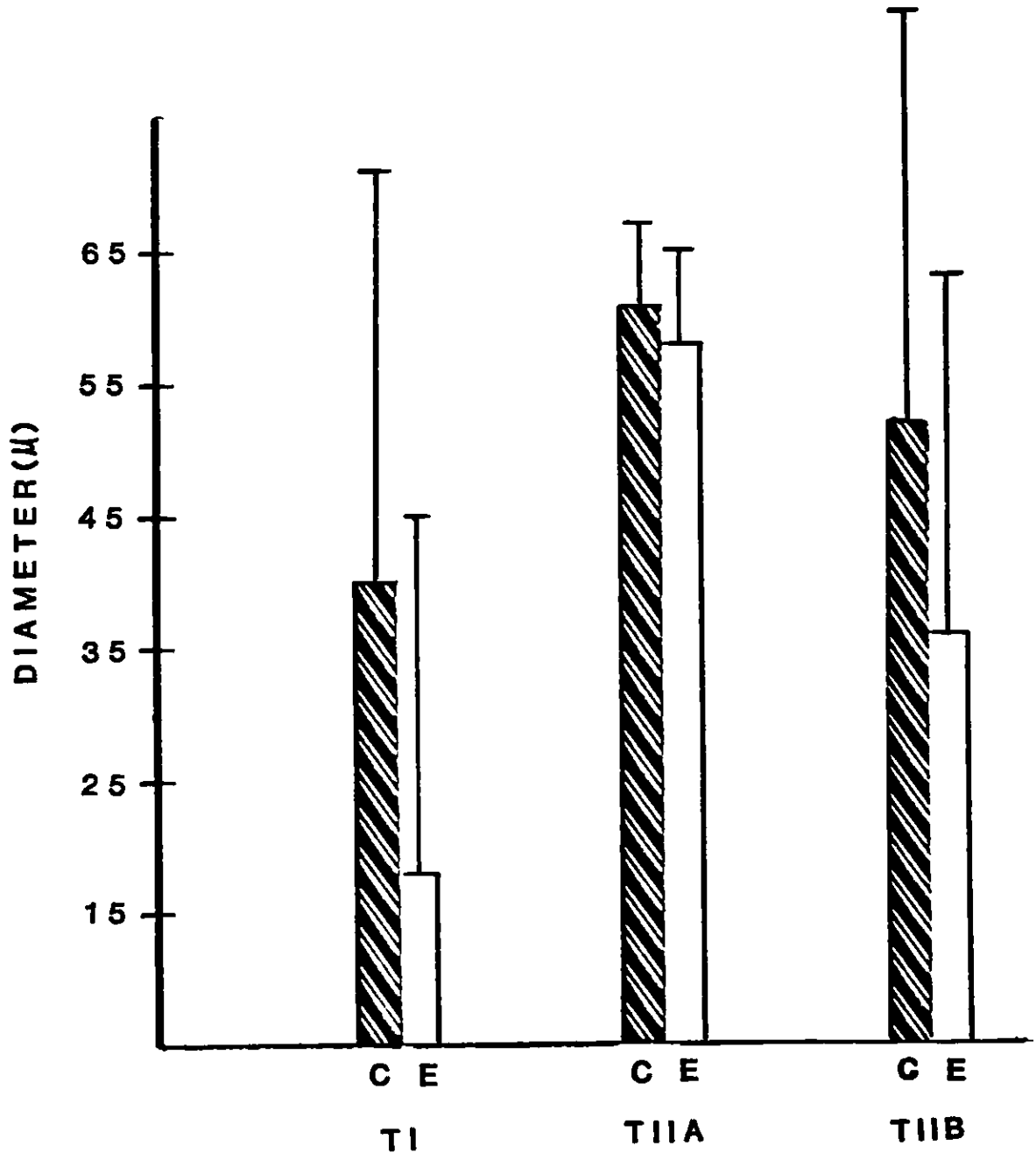


Figure 15: Mean fiber diameters: control and experimental Groups (20 days). Error bars indicate standard deviation.

MEAN FIBER DIAMETER

20 DAYS

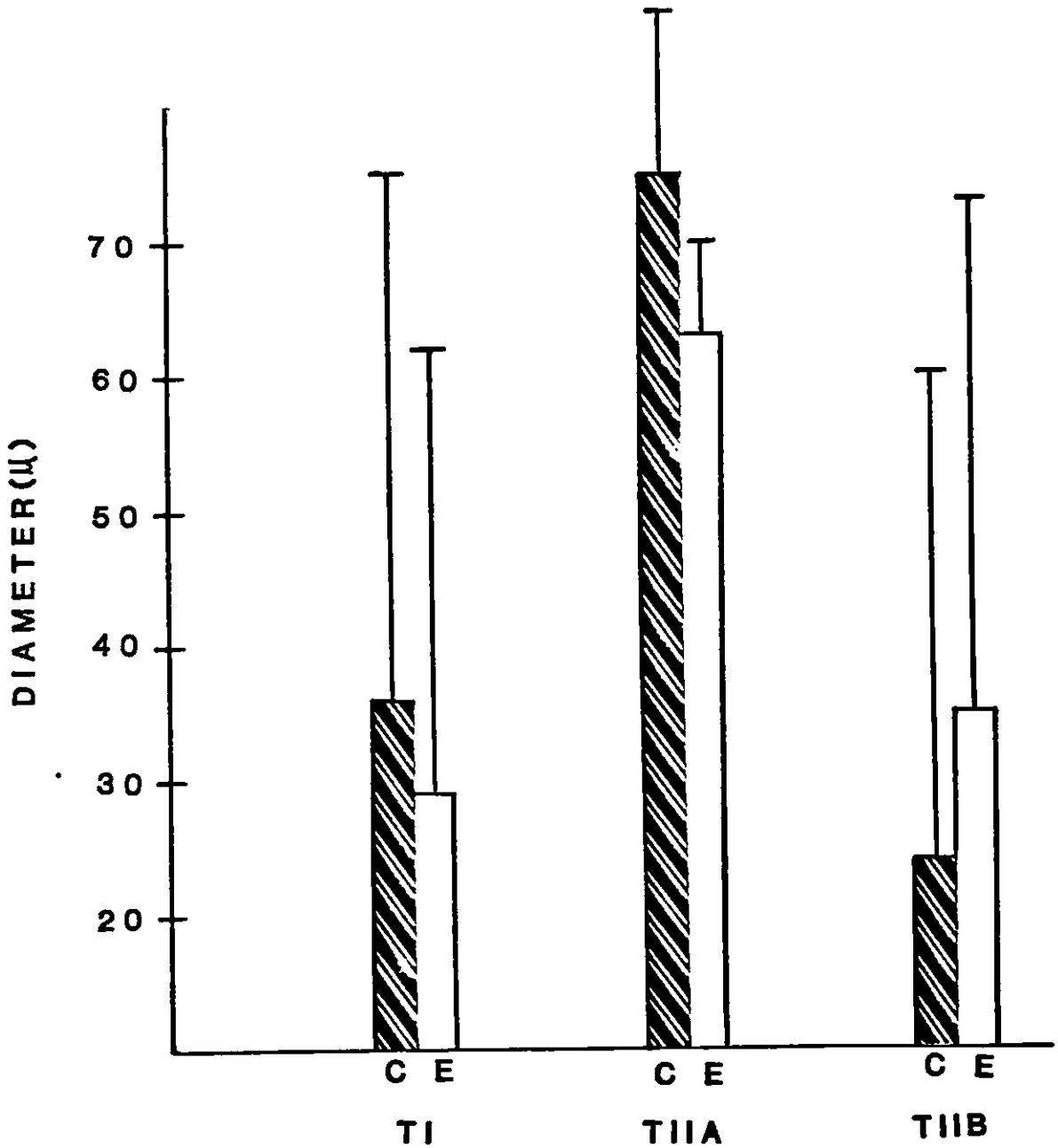


TABLE VIII

FIBER DIAMETERS
CONTROL AND EXPERIMENTAL GROUPS (10 AND 20 DAYS)

10 DAYS

Variable	Control		Experimental		Test $U_1 = U_2$		
	$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
Type I	50.83	25.32	17.76	27.5	2.17	10	NS
Type II A	63.14	6.09	58.91	6.54	1.16	10	NS
Type II B	63.01	9.68	35.58	28.14	2.28	10	$p<0.05$

20 DAYS

Variable	Control		Experimental		Test $U_1 = U_2$		
	$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
Type I	35.77	39.23	37.30	29.68	0.08	10	NS
Type II A	75.26	12.31	62.82	6.79	2.17	10	NS
Type II B	23.53	36.73	43.27	35.26	0.95	10	NS

α = Level of Significance

TABLE IX

FIBER DIAMETERS WITHIN
CONTROL GROUPS (10 AND 20 DAYS)

10 DAYS

Type I		Type II A		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
50.83	25.32	63.14	6.09	1.16	10	NS

Type I		Type II B		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
50.83	25.32	63.01	9.12	1.10	10	NS

Type II A		Type II B		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
63.14	6.09	63.01	9.68	0.03	10	NS

20 DAYS

Type I		Type II A		Test $U_1 = U_2$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
35.77	39.23	75.26	12.31	2.35	10	$p < 0.05$

Type I		Type II B		Test $U_1 = U_2$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
35.77	39.23	23.53	36.73	0.55	10	NS

Type II A		Type II B		Test $U_1 = U_2$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
74.26	12.31	23.53	36.73	3.27	10	$p < 0.01$

α = Level of Significance

TABLE X

FIBER DIAMETERS WITHIN
EXPERIMENTAL GROUPS (10 AND 20 DAYS)

10 DAYS

Type I		Type II A		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
17.76	27.5	58.91	6.54	3.56	10	$p<0.01$

Type I		Type II B		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
17.76	27.5	35.58	28.14	1.10	10	NS

Type II A		Type II B		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
58.91	6.54	35.58	28.14	1.97	10	NS

20 DAYS

Type I		Type II A		Test $U_3 = U_4$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
37.30	29.68	6.5	6.79	2.23	10	$p<0.05$

Type I		Type II B		Test $U_3 = U_4$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
37.30	29.68	43.27	35.26	0.32	10	NS

Type II A		Type II B		Test $U_3 = U_4$		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
5.5	6.79	43.27	35.26	1.48	10	NS

α = Level of Significance

TABLE XI

TOTAL NUMBER OF FIBERS
CONTROL AND EXPERIMENTAL GROUPS (10 AND 20 DAYS)

Control (10D)		Experimental (10D)		Test $U_1 = U_2$		
$\bar{X}_1$	SD_1	$\bar{X}_2$	SD_2	t	df	α
159.50	36.80	191.83	48.56	1.30	10	NS
Control (20D)		**Experimental (20D)**		**Test $U_3 = U_4$**		
$\bar{X}_3$	SD_3	$\bar{X}_4$	SD_4	t	df	α
119	29.26	145.50	24.12	1.71	10	NS
Control (10D)		**Control (20D)**		**Test $U_1 = U_3$**		
$\bar{X}_1$	SD_1	$\bar{X}_3$	SD_3	t	df	α
159.50	36.80	119	29.26	2.11	10	NS
Experimental (10D)		**Experimental (20D)**		**Test: $U_2 = U_4$**		
$\bar{X}_2$	SD_2	$\bar{X}_4$	SD_4	t	df	α
191.83	48.56	145.50	24.12	2.00	10	NS

α = Level of Significance

DISCUSSION

Skeletal muscle fibers have many components which are capable of adaptive changes in response to altered functional demands. A change in the number of sarcomeres in series seems to be the way by which a muscle adjusts to alterations in its functional length (Tabary et al., 1972; Williams and Goldspink, 1973). Further, muscle fiber size is known to be affected by the level of nutrition. Goldspink and Ward (1978) studied the response of different fiber types to partial starvation. Results showed a decrease in size, the greatest being in the ATPase high fibers. These same authors have also demonstrated hypertrophy of all three major fiber types in response to high intensity exercise (weight lifting). The changes were found to be completely reversible following recovery. The reason for undertaking the present study was to elucidate the experimental effects of mandibular advancement on the lateral pterygoid muscle by means of a stimulated functional appliance. In such a model, the muscle is shortened while still functioning, similar to the case during the clinical application of functional treatment. The experimental methodology focuses on changes that would occur within the muscle due to adaptational responses to altered functions.

Young rats (20 days old) were used to carry out the experiment. It has been shown that the greatest increase in fiber diameter and changes within fiber types occur during the first three weeks after

birth (Williams and Goldspink, 1971; Curless and Nelson, 1976). The fact that such data which are related to normal development in the young rats are available made it possible to compare between normal occurrences of growth and development and changes due to altered functional demands. The usual way of distinguishing between different fiber types is based on their histochemical staining for myosin ATPase after preincubation at acid or alkaline pH. Gollnick et al (1983) have suggested that histochemical techniques that differentiate among the different myosin ATPase isoenzymes can supply more objective ways of identifying different fiber types in the muscles than metabolic enzymes, since the ATPase reaction intensity patterns for the fibers are discrete and are less influenced by contractile behavior. Due to the proven validity of this technique, this was the method of choice for distinguishing between fiber types in the present study.

The appliances which were designed to achieve a 3 mm advancement of the lower jaw, had been tested previously to evaluate degree of interference with normal feeding behavior. Although it had been observed that consumption of food was possible, comparisons between control and experimental groups following treatment showed a reduced weight gain in the animals with anterior displacement of the mandible. This difference was statistically significant ($p < 0.01$). Biopsies of the soleus muscle (also used as reference for the staining procedures) of a control and experimental rat were analyzed to evaluate whether weight differences were significant factors

contributing to the differences observed in the lateral pterygoid muscle between control and experimental groups. However, there were no significant differences between the control and experimental soleus muscles with regard to fiber diameter or number of fibers. Of course, this conclusion is based on a very limited sample and it must be remembered that there are data to show that muscle fiber diameters are greatly influenced by nutritional factors (Goldspink and Ward, 1978).

Three types of fibers were identified in the lateral pterygoid muscles of all control and experimental animals. Type I fibers, which are slow contracting, resistant to fatigue and adapted for tonic use, as in postural maintenance. Type II B is fast fatiguable during repetitive activation and is suited for rapid, powerful contractions, while Type II A is resistant to fatigue. These characteristics have also been demonstrated in studies done on the histochemical properties of masticatory muscles (Hiraiwa, 1978; Rokx et al., 1984). They show that rat masticatory muscles, including the lateral pterygoid, are composed of mostly phasic type fibers (II A and II B) and only a small number of fibers of the tonic type (I). In my study, high ATPase fibers were found to be predominant in both control and experimental groups at 10 days as well as 20 days. The differences between types I and II were statistically significant ($p < 0.01$). The phasic contractions of masticatory muscles in small animals like rats, correspond well with the rapid jaw movements in eating (Hiraiwa, 1978).

The results of my experiment show a significant decrease of type II A fibers in both control and experimental groups ($p < 0.01$) with increasing age. It is also important to note that at the same time, the mean values for type I fibers showed an increase in number although they did not reach levels of statistic significance. Kugelberg (1975) examined changes in rat soleus motor units during growth. The muscle showed a considerable number of type II fibers (darkly stained) at 5 weeks. At adult age, the type II fiber units, capable of maintaining repetitive phasic contractions, were replaced by slow type I units, which are more efficient in maintaining sustained contractions. It seems that motoneurones are most influencial in changing the fibers in a unit from fast to slow twitch fibers in the process of growth and development, to adjust the muscle to its usage.

One parameter that is quite susceptible to changes during growth (Williams and Goldspink, 1971) and to altered function (Williams and Goldspink, 1978) is muscle fiber size. In my study statistical analysis only showed a significant decrease in diameter of type II B fibers between the control and experimental groups at 10 days. Types I and II A fibers showed no significant differences between treated and untreated animals. Although not likely, a reason could be that only fibers in one section per animal were measured. This reduces the number of examined fibers and consequently limits the areas along the muscle from which measurements are taken.

The sarcomere numbers were not studied in this experiment. Oudet and Petrovic (1978) inserted a postural hyperpropulsor in young rats and counted the number of sarcomeres in series in the lateral pterygoid muscle. The data showed a 25% shortening of the muscle while simultaneously the number of sarcomeres in series decreased. The result also showed that the amount of anterior displacement of the mandible was an important factor. The greater the magnitude of deviation, the greater the decrease in the number of sarcomeres. These findings are important examples of how readily adaptational changes occur in fiber size as a consequence of functional alterations of the muscle.

Comparisons of fiber diameters within control and experimental groups showed type II A fibers to be significantly larger than the type I fibers. Armstrong and Phelps (1984) concluded from their studies on muscle fiber composition of the rat hindlimb, that the relative sizes of the fibers of each type vary both within and among the different muscles. For example, in the deep red portions within muscles, both the slow oxidative (Type I) and fast oxidative-glycolitic (Type II A) fibers were generally larger than those in the superficial white parts of the same muscles. Similarly, the deep red extensor muscles (soleus and vastus intermedius) had the largest SO and FOG fibers in their respective groups. It is known that muscle fiber diameter is closely related to the forces the fibers are required to generate (Burke, 1981; Saltin and Gollnick, 1983). Increased usage induces relative fiber hypertrophy (Ianuzzo

et al., 1976), and disuse causes atrophy (Booth, 1977). From these associations follows that since type II A fibers in the lateral pterygoid muscle of the rat are continuously in use in the different mandibular movements, they are larger than the less active Type I fibers.

The difference in number of fibers between treated and untreated groups did not show statistical significance. This is in agreement with other data presented by other investigators. For instance, Goldspink and Ward (1979), reported that the number of fibers is not affected by either undernutrition or exercise.

Although most of the data presented did not show statistical significance and could be explained as normal occurrences of growth and development, they provide baseline information for further experimental studies. Some information is available on the histochemical profile of masticatory muscles in rats, but much more data are necessary, both normative and in response to functional alteration.

Transitory changes with regard to transformation of muscle fiber types or alterations in fiber diameters could be expected in the lateral pterygoid muscle as a result of adaptation. The fact that only minor differences were detected between control and experimental animals does not necessarily mean that changes did not occur within the muscle. The immediate response to altered function is morphological changes in the muscle. This is followed by skeletal responses. However, if the altered function is maintained

adaptation occurs in both muscle and bone. Given enough time the muscle may show complete structural adaptation. Thus, changes induced by altered function are only transitory. Similar adaptational responses were observed by Oudet and Petrovic (1978), with regard to the number of sarcomeres in the lateral pterygoid during functional treatment. The number decreased during the first weeks while from the 21st to the 28th day of treatment this trend reversed and the number of sarcomeres increased. The clinical treatment of class II maloclussions using an orthopedic appliance to produce anterior displacement of the mandible is usually carried out in young patients (7 to 12 yrs old). During this stage growth is still occurring throughout the craniofacial area. This growth is accomplished by remodeling (bone deposition and resorption) and movements in which the separation of bones, create spaces within which enlargement of each of the separate bones takes place. Thus, it is important to point out that during mandibular advancement contractions of the lateral pterygoid muscle would have an effect beyond that on the condylar cartilage (Petrovic and Stutzmann, 1972). It would also influence development of the whole ramus and consequently, the rest of the craniofacial skeleton due to the differential growth occurring in the various bones.

The appliance used in this study was designed to achieve a 3 mm mandibular advancement, and therefore, a 3 mm shortening of the lateral pterygoid muscle. This represents a shortening of about 10%. In a human, the range of advancement is 4-5-6-7 mm. The

muscle shortening in the therapeutic situation is probably in the range of 8 to 10% and therefore it is comparable to that used in this experiment. In a small animal like the rat, the relatively large mandibular displacement probably had a negative effect in normal food intake. It has also been suggested that if the anterior displacement of the mandible is too large, there may be a drop in muscular tension due to an excessive shortening of the muscle which may cause a decrease in the growth rate of the condylar cartilage (Petrovic et al., 1975).

Further studies should be carried out taking into consideration factors discussed. This will help clarify the mechanism by which the lateral pterygoid muscle is involved in the treatment response with so called functional appliances.

SUMMARY AND CONCLUSIONS

Many investigations have focused on the nature of muscle adaptation in response to functional alterations. However, more information is necessary regarding the effects of functional orthodontic appliance therapy on muscles of mastication. The objective of this experimental study was to investigate the response of the lateral pterygoid muscle to changes in its functional length during mandibular advancement induced by functional appliance treatment. The following is a summary of the results obtained from this study:

1. Using the histochemical method of myosin ATPase, three types of muscle fibers could be identified in the lateral pterygoid muscles with and without mandibular advancement: type I, type II A and type II B.
2. Muscle fiber counts showed that the normal lateral pterygoid muscle is predominantly composed of ATPase-high fibers (type II A). This pattern also is characteristic of shortened muscle.
3. Type II A fibers showed a significant decrease in both control ($p < 0.01$) and experimental ($p < 0.05$) groups with age.
4. Type II B fibers showed a significant decrease ($p < 0.05$) in diameter in the experimental group at 10 days when compared to the control group.

5. Morphometric measurements indicate type II A fibers to be significantly larger in diameter than type I fibers in both control and experimental groups.
6. No significant differences were found in the number of fibers between experimental and control groups.

The process of growth and patterns of use (either load or inactivity) have effects in establishing, maintaining and changing metabolic characteristics of skeletal muscle. The ability of the muscle to adjust its size to physiological demands is demonstrated by changes in fiber cross-sectional area that occur during normal growth and development and in the adult and growing animal in response to alterations in activity pattern. Such changes in muscle length are the result of a longitudinal growth produced by the addition of sarcomeres to the ends of the muscle fibers.

With changes in muscle fiber size, there is also a change in the fiber types characterized by changes in contractile properties and in the way that a fiber is utilized under different conditions.

During maturation after birth, there is an increase in slow twitch fibers in response to endurance type activity and the appearance of a greater percentage of fast twitch fibers in those activities where rapid repetitive contractions are required.

In my study, adaptive responses of skeletal muscle to altered function appeared to be only transitory (type II B fibers were significantly reduced in diameter in treated animals at 10 days). When the lateral pterygoid muscle functions in a shortened position

induced by anterior displacement of the mandible, changes occur in the muscle before skeletal responses. Due to continuous growth during treatment, the distance between the condylar and pterygoid process will increase and thus the muscle must continuously adjust. It is concluded that similarities in the lateral pterygoid muscle in treated and untreated animals is due to skeletal growth responses that occurred with and without treatment. Further, this study allowed comparisons with other investigations on normal postnatal developmental patterns of skeletal muscle.

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