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Fiber-type differences in masseter muscle associated with different facial morphologies

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Abstract

Background—The influence of muscle forces and associated physiologic behaviors on dental and skeletal development is well recognized but difficult to quantify because of the limited understanding of the interrelationships between physiologic and other mechanisms during growth.

Methods—The purpose of this study was to characterize fiber-type composition of masseter muscle in 44 subjects during surgical correction of malocclusion. Four fiber types were identified after immunostaining of biopsy sections with myosin heavy chain-specific antibodies, and the average fiber diameter and percentage of muscle occupancy of the fiber types were determined in each of 6 subject groups (Class II or Class III and open bite, normal bite, or deepbite). A $2 \times 3 \times 4$ analysis of variance was used to determine significant differences between mean areas for fiber types, vertical relationships, and sagittal relationships.

Results—There were significant differences in percentage of occupancy of fiber types in masseter muscle in bite groups with different vertical dimensions. Type I fiber occupancy increased in open bites, and conversely, type II fiber occupancy increased in deepbites. The association between sagittal jaw relationships and mean fiber area was less strong, but, in the Class III group, the average fiber area was significantly different between the open bite, normal bite, and deepbite subjects. In the Class III subjects, type I and I/II hybrid fiber areas were greatly increased in subjects with deepbite.

Conclusions—Given the variation between subjects in fiber areas and fiber numbers, larger subject populations will be needed to demonstrate more significant associations between sagittal

relationships and muscle composition. However, the robust influence of jaw-closing muscles on vertical dimension allowed us to conclude that vertical bite characteristics vary according to the fiber type composition of masseter muscle.

Subjects undergoing orthognathic surgery to correct skeletal and dental relationships are characterized by diverse craniofacial morphologies. These morphologies are not craniofacial anomalies or the result of recognized genetic syndromes but, rather, are extreme variations from the “normal” range that are severe enough to require surgical correction. Growth in orthognathic surgery patients clearly deviates significantly from a normative pattern, and these patients could be considered to have a craniofacial growth disturbance. These patterns have been characterized (on the basis of vertical dimension) into 2 extreme archetypes: the long-faced, open bite pattern and the short-faced, deepbite pattern.¹ After much investigation, the etiology of extreme facial patterns remains an enigma, because of a limited understanding of the basic physiologic (functional) and genetic mechanisms controlling skeletal growth and adaptation.^{2,3}

Sassouni¹ outlined the concept that vertical alignment (and subsequent force) of jaw-closing muscles directed skeletal growth toward a shallow mandibular plane angle, an acute gonial angle, and deepbite, whereas obliquely aligned jaw-closing muscles (with subsequent diminished force) permitted a steep mandibular plane, an obtuse gonial angle, and open bite. Bite force studies have documented diminished occlusal force at the molar occlusal plane in long-faced adults.⁴ These force differences might not be due to intrinsic muscle differences but, rather, to mechanical advantage loss in obliquely applied force.⁵ Others have imaged muscle to determine overall size and orientation using a variety of techniques, including cephalometrics,⁵ computed tomography,⁶ ultrasound,⁷ and magnetic resonance imaging (MRI),^{8,9} but they do not agree with regard to muscle size, orientation, and craniofacial form.

Because the relationship between muscle architecture and jaw growth is complex, an important aspect is the intrinsic composition of muscle in terms of its fiber types. Skeletal muscle is composed of a variety of fiber types with different functional and histologic characteristics. For example, postural muscles are composed mostly of type I fibers (fatigue resistant, slow contracting), whereas muscles used in rapid locomotion have higher proportions of type II fibers (fast contracting, relatively fatigable). Some human cranial muscles, including the jaw-closers, are very different in fiber-type composition compared with skeletal muscle from the limbs or abdomen. Using the isoforms of myosin (the major motor protein in these fibers) as the discriminator, we can currently characterize 8 fiber types in masticatory muscles, whereas limb muscle contains only 3 main types.¹⁰ In addition, human masseter shows wide individual variations in fiber-type composition, as demonstrated in biopsy studies.¹⁰⁻¹²

An important clinical consideration is whether this variability in masseter muscle composition is related to differences in craniofacial form. Previous studies based on small sample sizes, in which a less-discriminating histologic technique for identifying fiber types was used, demonstrated positive correlations¹³ but are too limited in scope to draw firm conclusions. Although some recent MRI studies have attempted to determine fiber-type composition by estimating tissue inorganic phosphate/phosphocreatine ratios, inaccuracy of imaging whole muscle with this technology is a significant shortcoming.¹⁴ Additional complications with imaging jaw-closing muscles are aberrant signals originating from the bone closely approximating the muscle and differences in fiber-type composition in comparison with skeletal muscles previously imaged with MRI.^{10,15}

In this study, we investigated the relationship between masseter muscle fiber-type composition and craniofacial form. We sampled masseter muscle from a large number of orthognathic surgery patients at the time of operation to determine fiber-type composition and compared this result with the Angle classification and vertical dimension for each subject.

MATERIAL AND METHODS

Masseter muscle biopsies were collected from a consistent site on the deep surface of the superficial layer of the left masseter muscle on the anterior border 3 to 4 cm above the mandibular angle in patients (mean age 28 years) undergoing a variety of orthognathic surgery procedures at the University of Lille. One surgeon (J.F.) excised the tissue, and a second (G.R.) collected information regarding clinical diagnosis and surgical procedures. Subject participation was in accordance with the research ethics committee of the University Hospital of Lille and with French patient privacy regulations. Limb muscle samples used as an internal “reference” for the fiber-type analysis came from patients undergoing soft tumor resections at Guy’s Hospital London, where a separate informed consent procedure was conducted for these subjects, with permission from Guy’s research ethics committee. The size of the biopsy was approximately 0.5 cm³. Tissue was snap-frozen, cryosectioned serially at 10 µm, and sections were mounted on glass microscope slides for immunostaining (indirect immunoperoxidase method) with antibodies specific for myosin heavy chain (MyHC) isoforms. These antibodies were specific for MyHC isoforms type I (BA-F8), all type II (MY-32), type IIA only (SC-71), neonatal (a polyclonal antibody prepared by A.R.) and α-cardiac (MAS 366) (also termed *atrial myosin*) and have been described previously.^{10,16} Batches of serial sections from several masseter biopsies were always stained with sections of at least one reference muscle, in many cases on the same slide, for comparison.

The panel of antibodies used permitted identification of 8 fiber types in masseter and the types present in the reference muscles, although these types were subsequently collapsed into 4 groups for statistical analysis as follows: type I: containing only type I MyHC; type II: containing only type IIA and/or IIX MyHC; type I/II hybrid fibers: containing both type I and II isoforms; and type neonatal/atrial: fibers that contained the neonatal or α-cardiac isoform in combination with other type I and II isoforms. For fiber type classification, only series with consistent antibody reactions from all stains and acceptable morphology of muscle fibers were used (ie, excluding any areas showing signs of handling or processing damage, such as ice crystal artifact or folds). A specific area was identified on each of the serially immunostained sections that was clearly in transverse section and had both adequate morphology and staining to type the fibers. This area was photographed in all relevant stained sections, so that individual fibers could be identified on serial images. All fibers within the selected areas were type-classified by 1 operator (ie, identified as I, I/II hybrid, II, or neonatal/atrial), and this classification was checked by a second operator before their cross-sectional areas were measured with image-analysis software.¹⁰ The area of each identified fiber on 1 stain from the series of sections was displayed as a digital image and sharpened, and its outer border traced with a VIDS-V image-analysis system (Ai, Cambridge, United Kingdom) linked to a Nikon Labophot microscope (Nikon, Tokyo, Japan). If the composition was uniform across the biopsy, only 1 area was analyzed; if there was any heterogeneity, 2 or more areas were analyzed to obtain a more representative sample. In general, every fiber with adequate staining and morphology was included in the analysis of a given area; only occasional fibers that were damaged were excluded. Tests for measurement error included intrarater reliability in determination of fiber area (by repeating morphometric tracing of all fiber areas in 1 biopsy by 1 examiner), which resulted in an R^2

value of 0.9452, and interrater reliability in determination of fiber area error, which resulted in an R^2 value of 0.9752.

An orthodontic diagnosis was made before surgery. To test the reproducibility of diagnosis, 4 board-certified (American Board of Orthodontics) orthodontists on faculty at the University of Pittsburgh were asked to classify pretreatment cephalometric and pano-metric radiographs for the following diagnostic criteria: Angle Class II or III (the sagittal relationship of the molars), and open bite, deepbite, or normal bite (the vertical relationship of the anterior bite). Classifications by these orthodontists matched the original presurgical diagnoses. If there was disagreement regarding the orthodontic classification, we were prepared to remove the subject from the study, but disagreement did not occur. Cephalometrics were not necessary, because of the obvious skeletal and dental discrepancies, which were typically deemed severe.

Morphometric analyses of fiber types and orthodontic classifications were carried out independently so as not to induce bias. Data for the subjects were analyzed with an analysis of variance (ANOVA) for a mixed between–within $2 \times 3 \times 4$ design, whereby class and bite were the between factors and fiber type the within factor ($2 \times 3 \times 4$ design = Class II or III relationship $\times$ open bite, normal bite, or deepbite $\times$ I, I/II hybrid, II, or neonatal/atrial fiber type). This analysis aims to show how each entity as a group varies compares with other groups (ie, whether the variations in the 4 fiber types, as a group, are significantly different in open bite versus normal bite versus deepbite, or significantly different in Class II versus Class III malocclusions).

RESULTS

General characteristics of biopsies and their fiber types

Of the tissue we collected, 2 upper limb, 8 lower limb, and 44 masseter muscle samples were of adequate quality to be sectioned, stained, and analyzed for fiber types and mean fiber cross-sectional area. The minimum number of fibers analyzed in a single masseter biopsy was 47 fibers in 1 area, but when there was heterogeneity across the biopsy, 2 or more areas were analyzed to give a representative sample. Overall, the mean number of fibers analyzed was 156 in masseter but only 77 in limb samples, which showed less heterogeneity. For each study subject, the orthodontic classification of malocclusion, the vertical dimension of bite, and the mean fiber area and number by fiber type are summarized in Table I. From these data, an additional calculation was performed to determine the percentage of total area of muscle tissue occupied by each fiber-type class. The mean fiber area was multiplied by the total number of fibers for each class to determine the total area occupied by class. From these total areas, the percent occupancy for each class was determined (Fig 1).

The limb samples, which were the internal reference for the fiber-typing methodology, showed the expected fiber type composition: approximately 40% type I fibers, nearly 60% type II fibers, few hybrid I/II fibers (0%-8%), and none in the neonatal/atrial group. An example is shown in Figure 2. Masseter fibers were, on average, smaller than their limb counterparts, especially for type II fibers (mean equivalent diameters $53.6 \mu\text{m}$ and $32.4 \mu\text{m}$ for types I and II, respectively). As expected, in masseter samples, the proportion of hybrid I/II fibers was generally higher, and there was a significant proportion of neonatal/atrial fibers (Fig 3). The presence of these hybrid and neonatal/atrial fibers is also reflected in the “occupancy” values, which take into account both fiber area and frequency, as shown in Figure 1.

Statistical analysis

The ANOVA with the area measure as the dependent variable resulted in significant main effects for bite ($P = .0134$) and for fiber type ($P = .0004$) (Table II). A significant class $\times$ bite $\times$ fiber-type interaction was also found ($P = .0374$), but only in the Class III group. No other effects in the model were statistically significant ($P = .3640$).

Post hoc tests with Fisher's least significant difference (LSD) procedure found that all fiber-type area means, except those between type II and neonatal/atrial, differed significantly ($P = .0004$). Only open bite and deepbite area means were significantly different ($P = .0044$). Tests for simple main effects in the class $\times$ bite $\times$ fiber-type interaction found that the bite $\times$ fiber-type interaction was not significant in Class II patients ($P = .6621$) but was significant in Class III patients ($P = .0457$) (Fig 4, *a*).

For Class III patients, the differences among bite groups in area were significant only for type II ($P = .0024$) and type I/II hybrid ($P = .0024$) fiber types. Simple-simple main effects tests found that open bite and normal bite patients differed significantly (both $P = .0014$) from deepbite patients on the type II fiber-type area means. Open bite and normal bite means for type II areas did not differ from each other ($P = .4030$) in these Class III patients. The type I/II hybrid fiber-type areas also differed significantly between open bite and deepbite Class III patients ($P = .0014$), and between normal bite and deepbite ($P = .0054$). Again, normal bite and open bite group means did not differ significantly for this fiber type ($P = .4730$).

The ANOVA results for percent occupancy were also highly significant for the main effect of fiber type ($P = .0004$) (Table II). A significant bite $\times$ fiber-type interaction was also found ($P = .0164$) (Fig 4, *b*). No other effects in the model were significant ($P = .2964$).

The post hoc LSD tests for fiber-type percent occupancy differences found type I fibers to differ significantly from each of the other types ($P = .0014$). Type I/II hybrid fibers differed significantly from neonatal/atrial ($P = .0364$), and type II differed significantly from neonatal/atrial ($P = .0504$). Tests for simple main effects found that bite differences in percent occupancy occurred only within type II fibers ($P = .0006$). None of the other bite $\times$ fiber-type differences was significant ($P = .1046$). Tests for simple-simple main effects found type II fiber percent occupancy to differ significantly between open bite and deepbite patients ($P = .0004$), and between normal bite and deepbite patients ($P = .0154$). Open bite and normal bite type II fibers did not differ on percent occupancy ($P = .1540$).

DISCUSSION

We investigated variations in skeletal muscle fiber type in orthognathic surgery subjects classified according to sagittal and vertical jaw relationships. In previous studies, we classified masseter fibers into 8 types according to variations in the type and amount of MyHC.¹⁰ In the present study, we categorized fibers into only 4 groups according to both MyHC composition and associated physiologic characteristics. Physiologic studies have shown that type I fibers have slow shortening velocities, whereas type II fibers have more rapid shortening velocities, and this applies to masseter as in other muscles. However, type II fibers in masseter muscle have a substantially smaller mean area than type I fibers and therefore produce substantially less force. Type I/II hybrids share features of both Classes I and II and possess physiologic capacity intermediate between the fast and slow fiber types.¹⁷ Finally, the neonatal/atrial type contains fibers with unusual motor protein expression, whose physiologic properties are not understood because they have not yet been examined at the single-fiber level.

We stratified the subjects by clinical diagnosis and surgical repositioning during treatment into 6 groups as either Class II or III with a vertical bite dimension of open, normal, or deep. Subject groups were compared in terms of variation in average cross-sectional area of fibers and the percent of total muscle sample composition (percent occupancy) of fiber type. All statistical comparisons of fiber types with each other, either for cross-sectional area or percent occupancy, were highly significant, indicating that the 4 fiber types identified in the study were distinct entities in masseter muscle. In comparing fiber-type area with the subject groups, there was a significant interaction between malocclusion class and vertical bite dimension, but this interaction was significant only for the Class III groups. However, differences in fiber-type percent occupancy were significant in the different vertical bite dimensions but not for malocclusion class. These results are summarized in Table II.

ANOVA produces significant findings when a group of values as a whole varies significantly, and the nature of the significant variances of our data is shown in Figure 4. To take Figure 4, *a*, as an example, some of the fiber types for a given vertical bite dimension seem to be very similar numerically but nonetheless are significantly different because it is the way in which all 4 fiber types, as a group, vary across the bite dimensions that produces the significance. Hence, this study demonstrated that vertical bite characteristics vary with the fiber-type composition of masseter muscle.

One constant feature of masseter muscle is the predominance of type I fibers: they have the largest mean area and often are the most numerous type. At normal vertical dimension, type I fibers occupy approximately half the tissue volume of masseter muscle and type II fibers only approximately 15%. In open-bite subjects, there is little difference in the size and occupancy of type I fibers, but the occupancy of type II fibers drops to approximately 8%, with an increase in hybrid fibers. In deepbite subjects, there are significant changes in percent composition of all 3 of these fiber types; that is, type I and hybrid fibers are substantially decreased in occupancy and type II fibers substantially increased in occupancy. The neonatal/atral group typically occupies a modest area of masseter muscle. Although much speculation is possible regarding these variations, a striking observation is the nature of variation in the type II fiber group. In open-bite subjects, type II fibers occupy the smallest area of masseter muscle, but, at the other extreme, in deepbite subjects, they are approximately equal in occupancy to the type I fibers. This is best illustrated in Figure 5, in which 2 subjects with rather severe open bite and deepbite are compared. Here the deepbite subject had approximately 50% greater type II fiber occupancy in masseter muscle. Our discussion of the impact of average fiber area to sagittal relationships was limited to Class III subjects because statistical results found significant interactions between bite dimension and fiber areas for only this subject class. Average fiber area for the type II fibers was smaller in deepbite than in Class III subjects with normal bite dimension, but the overall muscle occupancy was greatly increased, meaning that the type II fibers were more abundant. Class III open-bite subjects also had type II fibers of smaller fiber area, but the number of these fibers was small, making their occupancy in the muscle minimal. Type I/II hybrid fibers varied accordingly because, as the percent occupancy of type II fibers increased, that of the type I/II hybrids decreased.

This variation between type II and type I/II hybrid fibers is consistent with current understanding of myosin transitions in skeletal muscle. Fiber transitions from one type to another are common occurrences in development, altered function demands, disease, and aging.¹⁸ In limb muscles, these transitions might be in the direction of slow type or fast type fibers in the sequence of I ↔ I/II ↔ II ↔ II/I ↔ II/I/II ↔ II/I/II/II.¹⁹ In this study, the transition has been simplified to I ↔ I/II ↔ II because we did not subclassify the type II fiber groups. In endurance training, as exemplified by extensive treadmill running in animals, there is a transition toward type I fibers, with a greater resistance to fatigue.²⁰ Resistance training, which demands significant force production against a load over short periods of time,

produces transitions to type II fibers, with increases in muscle mass and strength, but with greater susceptibility to fatigue.²¹ Inferring that similar adaptations occur in masseter muscle however, must take into account anatomic and functional differences. Resistance and endurance training studies usually describe changes in muscles of the upper or lower limb after extreme functional demands are placed on locomotion around a particular joint. The joint associated with masseter muscle is by no means simple and represents an articulation between 2 cartilaginous structures (the temporomandibular joints) and the dentition. Furthermore, humans are unlikely to engage in the same type of functional activities with their jaws that would lead to extreme transitions in fiber-type composition.

The finding that greater percentages of type II fibers are found in deepbite seems at first to be intuitive, but not all investigators have agreed with this association. A recent MRI study of masseter muscle has come to the opposite conclusion, that a possible increase in type I fibers is associated with a decrease in maxillary-to-mandibular divergence.¹⁴ MRI of muscle measures the relative amount of inorganic phosphate, phosphocreatine, and metabolically active forms of adenosine triphosphate. Variations in these metabolites reflect the metabolic nature of the muscle. When a muscle composed predominantly of type I fibers (the soleus muscle of the lower limb) is compared by MRI with other muscles of mixed-fiber composition, the soleus is found to have higher levels of inorganic phosphate. There are 2 main interpretations for this: it might indicate greater metabolic activity from cross-bridge cycling of myosin with actin, or it could be related to the much higher capacity for oxidative metabolism in type I fibers compared with type II in limb muscles. The finding that inorganic phosphate levels increase with decreases in maxillary-to-mandibular divergence thus might not mean that there is an increase in type I fibers¹⁴ but, rather, only that metabolic activity is increased or more oxidative.

The association of type I fibers with inorganic phosphate levels might be true for an archetypical slow muscle, the soleus, but might not hold true for highly adapted cranial muscles, such as the masseter. For example, a preliminary survey of 15 of our masseter samples showed a similarly high oxidative activity in all fibers, including type II, as shown by histochemical staining for the mitochondrial enzyme cytochrome oxidase. Only a few of the smallest-diameter masseter fibers were low in cytochrome oxidase activity, whereas in the limb reference samples type II fibers always had lower cytochrome oxidase staining. This is consistent with previous studies in which a higher oxidative activity and capillarization of masticatory muscles compared with limb muscles in man was reported.^{22,23} Any hypothesis regarding the etiology of open bite or deepbite must take into account differences in fiber type, especially the presence or absence of unusually small-sized type II fibers. It is unlikely that the increased amounts of type II fibers we found in our deepbite subjects would lead to increased basal metabolic activity in masseter muscle. The findings of Al-Farra et al¹⁴ might also not apply to our results, because of differences in subjects. Our subjects were recruited from an orthognathic surgery population, whereas Al-Farra et al¹⁴ imaged healthy volunteers who apparently had no orthodontic problems.

Two other important attributes regarding masseter activity are data ascertained from electromyography (EMG) recordings and bite-force measurements. Bite-force transducers determine the amount of force muscles transfer to the dentition, and EMG determines the characteristics and amount of electrical activity used in generating the force in muscle fibers. A combined fiber-type analysis, EMG, and bite force study has not yet been conducted, and the associations between fiber-type content and bite force have been described only once previously.²⁴ Here, increases in the amount and size of type II fibers in masseter muscle were associated with increases in average bite force.

Others have looked at craniofacial dimensions and bite force but without ascertaining fiber-type content of muscle. Long-faced subjects have less of a mechanical advantage during biting than normally proportioned ones, because of their more obtuse gonial angle and subsequent jaw divergence at the occlusal plane.²⁵ Yet this does not seem to fully explain decreases in bite force associated with open bites. Long-faced and normally proportioned children produce similar bite forces, which approximate the typical bite force of long-faced adults,²⁶ but the normal children at maturity develop bite forces that nearly double from adolescent levels. The inability of long-faced subjects to develop normal bite forces in adulthood cannot therefore be attributed to differences in skeletal relationships. This factor and the association of variations of fiber type with bite force²⁶ are strong evidence that muscle tissue regulates force levels at the occlusal plane, regardless of facial pattern. In an individual patient, the final outcome of growth is determined by the balance between the different elements. One of these elements is muscle activity, and the results of our study are interesting because they show a link between a vertical growth disturbance and a particular muscle fiber-type composition. Further study of the associations between EMG activity, bite force, fiber-type content, and craniofacial morphology will be necessary to understand vertical development of the face.

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Fig 1.

Mean percentages and standard deviations of total area of masseter sample occupied by each fiber class ("occupancy") for 6 masseter subject groups. *II*, Angle Class II; *III*, Angle Class III; *O*, open vertical bite; *N*, normal bite; *D*, deepbite.

Fig 2.

Immunostaining of serial sections of adductor magnus muscle biopsy. Panels show staining with **a**, anti-type II myosin, **b**, anti-type I, and **c**, anti-type IIA. Fiber types I, IIA, and IIX as labeled. *I/II hybrid fiber type.

Fig 3. Immunostaining of serial sections of masseter muscle biopsy. Antimyosin antibodies used are named on each panel. *Lower right panel* shows staining profiles for type classification. *Arrows* indicate some fibers of neonatal/atrial category: note that their reactivity with type I and II antibodies is variable, but they must react with either anti-neonatal or anti-atrial antibody to be classified as neonatal/atrial fiber type.

Fig 4.

Significant interactions found by ANOVA test of significance. Mean fiber areas differed with vertical bite dimension and Angle classification for Class III subjects only (a). Muscle occupancy for fiber type was significant for vertical bite dimension (b) but not for Angle classification.

Fig 5.

Lateral cephalograms of 2 representative subjects demonstrating extremes of vertical bite discrepancies in relation to fiber-type occupancy of their masseter muscle biopsies.

Table I

Mean fiber areas (μm), confidence intervals (CI), and number of fibers analyzed by fiber type for all subjects

Class	Bite	Type I fibers			Type I/II hybrid fibers			Type II fibers			Type neonatal/atrial fibers		
		Mean ϕ	CI	n	Mean ϕ	CI	n	Mean ϕ	CI	n	Mean ϕ	CI	n
2	Open	1568.6	177.0	43	1346.9	208.3	42	297.1	44.1	16	1288.9	365.8	24
2	Open	1829.3	248.3	58	2366.3	220.9	35	267.0		1	1382.3	203.1	4
2	Open	1413.9	113.8	54	1077.6	135.6	18	412.4	162.2	8	867.8	63.3	85
2	Open	2759.1	233.8	47	2407.9	523.0	10	2727.4	4045.0	3	1456.8	158.6	32
2	Open	2730.9	338.2	57	1976.1	250.8	24	182.3		1	643.0	90.1	78
2	Open	2525.2	236.3	47	1214.9	456.7	14	695.3	64.2	62	569.5	58.1	29
2	Open	1515.4	108.4	71	893.0	114.9	42	600.4	51.2	8	624.6	64.2	35
2	Open	1127.7	213.7	14	1095.5	99.3	53	317.8	27.3	103	444.6	74.8	25
2	Open	3484.5	487.0	42	2026.1	230.1	90	1007.7	490.8	24	590.8	135.8	35
2	Open	1150.6	84.2	82	1717.0	132.8	89			0	478.9		1
2	Open	1560.8	105.9	144	1860.6	289.4	42	528.0	48.3	117	1062.7	50.5	139
2	Normal	3593.1	340.8	23	2179.4	308.1	11	357.7	94.5	31	1498.0	316.8	24
2	Normal	2089.2	120.3	37	1205.4	355.9	11	649.8	108.3	50	584.4	108.7	14
2	Normal	2275.9	309.6	35	1798.3	270.7	41	416.9	235.7	2	984.3	108.8	27
2	Normal	3191.5	212.3	62	1241.1	230.9	10	1343.3	229.8	14	1105.9	214.5	19
2	Normal	2346.7	144.3	159	1859.9	198.4	105	1325.1	185.1	37	1076.8	332.1	9
2	Normal	2718.0	330.7	17	2276.5	384.3	12	575.8	108.7	39	1201.9	196.3	34
2	Normal	1795.9	135.5	53	1301.3	197.7	35	386.1	41.8	27	756.3	131.4	12
2	Deep	2967.8	326.1	26	2231.2	528.6	9	1125.0	417.7	14	1813.5	208.9	30
2	Deep	1542.9	164.5	53	1701.1	133.7	54	380.5	189.3	2	1069.4	26.7	2
2	Deep	2500.0	218.1	56	1911.9	510.7	11	970.6	127.4	128	1620.7	192.9	38
2	Deep	3592.2	723.4	22	3079.5	753.6	7	3184.0	270.2	40	1179.4	882.2	10
3	Open	1196.1	121.8	73	1389.9	116.0	106	565.6	467.1	7	780.6	70.6	69
3	Open	2201.2	222.6	68	1077.6	286.5	24	641.8	68.3	59	666.7	92.1	52
3	Open	2632.4	174.5	56			0			0	748.0	79.6	106
3	Open	2910.4	422.5	41	2248.3	763.9	5	1881.1	399.8	23	1247.9	109.3	6
3	Open	2747.1	364.4	30			0	521.5	186.4	3	1432.5	146.7	62
3	Open	2543.6	522.6	39	2441.3	376.0	20	1414.6	204.4	79	1208.9	244.7	44

Class	Bite	Type I fibers			Type I/II hybrid fibers			Type II fibers			Type neonatal/atrial fibers		
		Mean $\bar{Q}$	CI	n	Mean $\bar{Q}$	CI	n	Mean $\bar{Q}$	CI	n	Mean $\bar{Q}$	CI	n
3	Open	2387.1	210.2	54	2165.3	730.4	8	265.6	52.1	4	818.5	147.3	46
3	Open	1898.4	181.2	41	1321.8	155.0	60	820.0	145.7	12	669.7	145.4	5
3	Open	1895.4	145.2	55	621.5	71.7	15	470.2	48.0	44	609.0	49.2	42
3	Open	2219.4	152.2	115	1407.9	130.8	52	41.1		1	988.0	186.9	19
3	Open	1570.7	97.3	109	1390.8	93.1	95	548.4	56.9	49	495.4	292.0	2
3	Open	1962.6	111.1	93	1925.5	210.9	42	243.0	35.3	41	339.0	77.6	57
3	Open	1419.6	101.0	100	1069.9	93.9	123	333.6	76.9	24	453.3	72.0	14
3	Open	1861.4	188.0	44	1199.3	197.3	34	110.6	125.9	2	726.8	62.1	112
3	Normal	2347.1	135.3	85	1777.5	169.5	12	1601.8	171.9	31	665.8	58.6	50
3	Normal	2090.4	403.9	16	2753.6	930.8	9	908.0	133.9	21	2735.9		1
3	Normal	2027.9	194.0	44	1974.9	159.8	4	1614.8	278.8	26	1282.7	731.6	6
3	Normal	1026.3	147.9	23	715.8	88.4	40	411.7	25.5	51	482.6	125.6	2
3	Normal	1735.8	164.2	51	1705.5	308.4	26	452.2	115.7	29	976.9	78.7	35
3	Deep	3195.0	1214.5	9	3655.6	487.1	9	404.7	101.6	141	761.7	451.7	25
3	Deep	2721.2	138.3	70	2169.9	521.7	12	1521.3	134.3	123	1206.8	256.8	30
3	Deep	4611.1	465.3	4	4249.8	461.7	19			0	1440.0	186.6	43

Table II

Significance of interactions as determined by ANOVA

	Mean fiber areas	Percent occupancy, vertical dimension
Fiber type	<i>P</i> .0004	<i>P</i> .0004
Fiber type × class	NS	NS
Fiber type × bite	NS	<i>P</i> .0164
Fiber type × bite × class *	<i>P</i> .0374	NS

NS, Nonsignificant.

* Significance found for Class III subjects only.