Stiffness of the Inferior Oblique Neurofibrovascular Bundle

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Purpose. To assess the mechanical ability of the inferior oblique neurofibrovascular bundle (NFVB) to act as an ancillary origin for the inferior oblique muscle after anterior transposition.

Methods. Stress–strain relations and Young's modulus of elasticity, a measure of tissue stiffness, were determined for the NFVB in vitro, in situ, and in vivo in dynamic and static conditions. For comparison, similar studies were performed in vitro on the superior oblique tendon (SOT).

Results. Young's moduli for NFVB in situ (6.3 MPa [megapascals]) and in vivo (11.8 MPa) were approximately 2 and 4 times greater (P < 0.05), respectively, than those of isolated NFVB in vitro at 5% to 10% dynamic strain (3 MPa). In dynamic conditions, Young's moduli in vitro for the NFVB and the SOT were similar.

Conclusions. The NFVB is a biomaterial that has stiffness properties similar to the SOT. Within the range of forces typical of normal eye movements (79 to 393 mN), the NFVB alone can tolerate forces of 98 mN at 0% to 10% strain and 393 mN at 15% to 20% strain, based on dynamic in vitro analysis. The greater measured stiffness in situ and in vivo suggest that the NFVB in the intact orbit potentially has a resting strain of 15% to 20%, and additional tissues in parallel with the NFVB also contribute to total stiffness. These data support the hypothesis that the NFVB, acting alone or in concert with adjacent orbital tissues, may form an ancillary origin for the inferior oblique muscle after anterior transposition. Invest Ophthalmol Vis Sci. 1997;38:1314-1320.

Anterior transposition of the inferior oblique muscle has become a popular method for the correction of its overaction as well as dissociated hypertropia.1-3 After surgery, however, significant elevation deficiency and primary position hypertropia have been reported.4-6 To explain this effect, it has been suggested that the inferior oblique is converted from an elevator to a depressor after anterior transposition.7,8 Conversion is probably dependent on the formation of an ancillary origin for the inferior oblique from closely associated orbital tissues. The idea that orbital connective tissue may serve an accessory locomotor function in cooperation with the extraocular muscles is well established.9 For example, there is evidence that fibrous muscular pulleys within the orbit may serve as functional origins of the recti muscles in lieu of their anatomic origins.10,11 The orbital connections of the inferior oblique muscle have been well described.12,13 Although fibrous connections between the capsules of the inferior oblique and inferior rectus muscles (Lockwood's ligament, for example) could serve as, or contribute to, the ancillary origin of the inferior oblique,13 others disagree.14,15 Based on anatomic analysis, it has been proposed that an anteriorly displaced inferior oblique could be tethered to the posterior orbit by its neurofibrovascular bundle (NFVB).16,17 This could have the effect of changing the direction of the force of contraction generated by the inferior oblique muscle to convert the muscle from an elevator to a depressor (Fig. 1). For this to occur the orbital tissues forming such an ancillary origin must possess both an anatomically favorable position and mechanical properties sufficient to tolerate the forces generated by the extraocular muscles. To investigate the latter, we determined the stress–strain relations and stiffness of the NFVB.
FIGURE 1. Left eye as seen from below. (a) Normal relations among (A) inferior oblique; (B) neurofibrovascular bundle; (C) inferior rectus; and (D) maxillary bone. (b) Position of the inferior oblique muscle after anterior transposition. Arrows indicate possible force vectors generated by inferior oblique muscle contraction (from Weakley and Stager36).

and its orbital connections to the inferior oblique muscle.

METHODS

Stress-strain relations and Young’s modulus of elasticity were determined for the human NFVB from thawed fresh cadavers (containing no fixative) and in live subjects. Measurements were obtained from the NFVB in intact cadaver orbits (in situ), isolated from cadavers (in vitro), and intraoperatively (in vivo). For comparison, isolated cadaver superior oblique tendons were also evaluated in vitro. All investigations involving human subjects followed the tenets of the Declaration of Helsinki, parental informed consent was obtained after the nature and possible consequences of the study were explained, and all studies were approved by the institutional review board.

Background

Stress-strain relations and Young’s modulus of elasticity have been used to describe the mechanical stiffness of various tissues within the orbit.19–21 The basic principles are as follows. Force is expressed in Newton (N; kg·m/second²) or milli-Newton (mN; g·m/second²)—for example, a 5-g mass under the influence of gravity (9.812 m/second²) results in a force of approximately 49 mN. Force normalized to the tissue cross-sectional area (m²) defines stress (that is, N/m²). The relative change in length of a tissue, as a fraction or percentage of its resting or slack length in response to a given stress, defines strain (unitless). The slack length is defined as the length at which stress is slightly greater than zero. The slope of a tangent to the strain versus stress relation represents Young’s modulus of elasticity, which is expressed in megapascals: 10⁶(N/m²) = MPa. This modulus quantifies the stiffness of a tissue, or the change in stress that results from a given strain, and provides a convenient means of comparing stiffness between tissues.22 Force data are reported in mN; stress and stiffness data are reported in MPa.

In this study, static stress–strain was determined from manual changes of applied force or length, whereas dynamic stress–strain was determined from computer-controlled length changes of a given strain at a specified strain rate. In both cases, the maximum force, or length response, was used to construct the stress–strain relations. Young’s modulus was calculated as a tangent to the stress–strain relations.20,23 In none of our experiments was a tissue taken to failure.

Static Stress–Strain Relations

In the study in situ, six cadavers were thawed for immediate dissection. A hemostat, with one jaw tied to a calibrated Ohaus [Florham Park, NJ] spring scale with 4-0 silk suture, was clamped to the exposed NFVB in the intact orbit (Fig. 2). The NFVB was then cut distal to the hemostat; and force from 49 to 491 mN (5 to 50 g), in increments of approximately 50 to 100 mN, was applied manually by the spring scale in the plane of the vertical action of the inferior rectus muscle. Displacement in response to each increment was determined by noting the initial and final position of a knot tied in the silk suture relative to a fixed scale. Maximal displacements for this force range were ap-

FIGURE 2. In situ or in vivo, the neurofibrovascular bundle was connected to a spring gauge (not shown), cut distally, and stressed. Strain was determined from displacement of a knot tied in the suture: A = inferior oblique; B = neurofibrovascular bundle; C = inferior rectus; D = intermuscular connections.

approximately 2 mm. The contribution of the hemostat mass to the spring scale reading throughout the 2-mm displacement was determined in the absence of an NFVB. The hemostat was balanced over the operator’s hand so that, when rotated over this fulcrum, no more than 2.5 g of mass resulted. When measurements were made on NFVB, the hemostat was similarly balanced so that changes in mass could be calculated from this reference of 2.5 g throughout the displacement range.

In the study in vivo, intraoperative measurements were obtained by methods similar to those used for the studies in situ on four children undergoing denervation and extirpation procedures for recurrent overaction in the inferior oblique muscle.

In the study of static stress–strain in vitro, the NFVB was dissected in five cadavers and stored in physiologic saline at 4°C until analyzed. Each bundle was suspended vertically in a jacketed organ bath filled with 4-0 silk suture to a hook at the bottom of the bath and to a Grass FT03 isometric force transducer (Astro-Med, West Warwick, RI). The bundles were incubated at 25°C in physiologic saline of the following composition (in mM): NaCl, 120.5; KCl, 4.8; MgSO4, 1.2; NaH2PO4, 1.2; NaHCO3, 20.4; CaCl2(2 H2O), 1.6; glucose, 10; pyruvate, 1, pH 7.6. The transducers were mounted on a movable stage controlled by a manual micrometer to adjust and monitor tissue length, and output was recorded on a Grass Model 7D Polygraph (Astro-Med). Slack length was determined as the distance between the two silk ties when force output in response to stretch was slightly greater than zero. Each bundle was subjected to a series of increasing loads that ranged between 0 and 491 mN (0 to 50 g) in increments of approximately 50 to 100 mN. Length changes associated with each force level were determined from the micrometer so that tissue elongation could be determined as a function of increasing load. The static stress–strain properties of four superior oblique tendons were similarly obtained for comparison. The data for each bundle and tendon sample were fit with a parabola and the determinants used to predict stress in a range of 0% to 30% strain in 5% increments. The mean correlation (r) between the individual sample raw and fitted values for the NFVB was 0.997, and for the superior oblique tendon (SOT) was 0.995.

Dynamic Stress–Strain Relations

Because the eye is a highly mobile organ, we thought it important to determine the stress–strain relation in dynamic conditions in a probable range of physiologic strain. Ligaments and tendons typically have a strain range of 4% to 10%; therefore, we selected 10% as the upper limit of strain for our analysis.

Dynamic stress–strain measurements were obtained from force and position outputs of a servocontrolled galvanometer (model 300H; Cambridge Technologies, Waterton, MA) under computer control. Custom software controlled the rate and magnitude of displacement of either the NFVB or the SOTs. Force and length change signals were processed by Gould universal amplifiers and recorded on a Gould TA4000 Thermal Array recorder (Gould, Valley View, OH). Each sample of NFVB and SOT was suspended vertically in a jacketed organ bath filled with physiologic saline between a clamp and the servo-arm with 4-0 silk suture. The tissue samples were subjected to a fixed strain (1.25%) in a range of strain rates that varied from 13% to 100% per second, and then were subjected to strains that varied from 1.25% to 10% at a fixed strain rate of 100% per second. For example, a 10% strain at 100%/second indicates that a bundle that is 20 mm long would be stretched 2 mm at a rate of 20 mm/second.

Data Analysis

Fractional strain was determined by dividing the length change associated with each force level by the slack length; percentage of strain was determined by multiplying fractional strain by 100%. Stress was ex-
Neurofibrovascular Bundle Stiffness

1.0 - 0.9 - 0.8 - 0.7 - 0.6 - 0.5 - 0.4 - 0.3 - 0.2 - 0.1 - 0.0 - -0.1 -
• - in vivo
D - in situ
• - dynamic in vitro
o - static in vitro

0 10 20 30 40
Strain [%]

FIGURE 3. Stress–strain data for the neurofibrovascular bundle from four methods of measurement. Data from each sample were fitted by a parabolic function (solid line) or linear regression (broken line). The slope tangential to each curve is Young's modulus for determining tissue stiffness.

pressed in megapascals. At the conclusion of each in vitro experiment, the final tissue length was measured, the sample gently blotted dry, and its mass determined. The cross-sectional area of the NFVB was determined by dividing mass by the product of slack length times a density of 1.13 g/cm³. Density of the NFVB was determined by the displacement method. Briefly, the mass (in grams) of a bundle divided by the volume of water it displaced (in milliliters) when completely immersed yielded the density (g/ml = g/cm³). Density of the NFVB was determined by the displacement method. For some tissues, cross-sectional area was determined by measuring the diameter of the tissue with an engineer's micrometer and using the equation, area = πr², assuming cylindrical geometry. This method yielded a very similar mean cross-sectional area for both the NFVB (0.0082 versus 0.0079 cm²; n = 8) and the SOT (0.035 versus 0.039 cm²; n = 5) compared with that obtained from the mass-over-length calculation. A value of 1.12 g/cm³ was used for the density of the tendon. For the in vitro static stress–strain relations, Young's modulus of elasticity for each bundle and tendon was determined by linear regression of tangents to the predicted stress–strain relations at a 5%, to 10%, 10% to 15%, 15% to 20%, and 20% to 25% strain. For the dynamic stress–strain relations, the dynamic curves were extended by extrapolation, using a parabolic fit, and linear regressions were performed as described above. Young's modulus for the stress–strain relations in situ and in vivo were also derived from linear regression (Fig. 3). Young's modulus (in megapascals) was determined by multiplying the slope of each linear regression (megapascals per percentage point) by 100%. This is the same as dividing stress by fractional strain.

Statistics
The static and dynamic Young's moduli data were analyzed by a one-way analysis of variance for repeated measurements. The Neumann–Keuls post hoc test was applied to all significant main effects to determine differences between means. Student’s t-tests for independent samples were used to determine differences between means obtained by different methods of measurement (that used in vivo versus that used in situ). Differences were considered significant for \( P < 0.05 \). Data are presented as mean ± SE.

RESULTS
The objective of this study was to determine whether the NFVB was sufficiently stiff to serve as an ancillary origin for the inferior oblique muscle after anterior transposition. Stress–strain relations and Young's modulus, a measure of tissue stiffness, were determined for the NFVB in static and dynamic conditions in vitro and in static conditions in situ and in vivo. The SOT was similarly assessed in vitro for comparison. Morphologic characteristics of the NFVB and the SOT used in the in vitro measurements are summarized in Table 1. Note that the strain values presented as percentages can be converted to millimeters by multiplying the percentage times the appropriate mean length reported for the NFVB or the SOT in Table 1.

Representative stress–strain responses obtained for each of the different modes of measurement demonstrate how the data were fit by either linear regression or by a parabolic function (Fig. 3). Our measurement system consisted of two components in series:

<table>
<thead>
<tr>
<th>Tissue</th>
<th>( L_{slack} ) (mm)</th>
<th>Mass (g)</th>
<th>Cross-Sectional Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neurofibrovascular Bundle (n = 14)</td>
<td>21.3 ± 1.3</td>
<td>17.6 ± 1.9</td>
<td>0.0073 ± 0.0005</td>
</tr>
<tr>
<td>Superior Oblique Tendon (n = 9)</td>
<td>19.2 ± 1.8</td>
<td>78.0 ± 11.9</td>
<td>0.0034 ± 0.0029</td>
</tr>
</tbody>
</table>

Values are mean ± SE.
the NFVB or the SOT and the 4-O suture. When two materials of different stiffness are arranged in series, total stress will be the same for both, but total strain will result from the sum of the individual material strains. To determine the strain attributable to the suture alone, its stress–strain relation was determined by subjecting it to the same absolute stress as was applied to the suture-plus-tissue during dynamic measurements. The suture was much stiffer than either the NFVB or the SOT, and therefore contributed minimally to total strain (Fig. 4). The Young’s modulus values presented here include both elements.

A comparison was drawn between the mean dynamic and static stress–strain relations determined in vitro for the NFVB and the SOT and the dynamic stress–strain relationship of 4-O suture (Fig. 4). Both the dynamic and static mean stiffness values for the SOT were less than the respective values for the NFVB, although for most comparisons these differences were not statistically significant. Strain rates from 13%/sec to 100%/sec had no effect on dynamic stiffness in either the NFVB or the SOT (data not shown). These results suggest that throughout the range of strain rates examined the stiffness of the NFVB has a very small viscous component, in that stiffness did not increase with increased strain.

To compare the dynamic and static stress–strain relations obtained in vitro, Young’s modulus of elasticity was determined on each tendon to four different strain ranges (Table 2). At each of these, dynamic NFVB stiffness was greater than static. In addition, the stiffness of the NFVB both in situ and in vivo at a 5% to 10% strain was greater than the dynamic stiffness in vitro at a comparable 5% to 10% strain (Fig. 5; Table 2). As the slopes of the lines indicate, it is clear that at strains up to 20% to 25%, stiffness in vivo and in situ would exceed stiffness in vitro. Stiffness in vivo was greater than that obtained in situ.

**DISCUSSION**

Anterior transposition of the inferior oblique muscle is thought to convert the muscle from an elevator to a depressor. This change in action suggests that an ancillary origin becomes functional after transposition. Elevation of the healthy eye requires activation of both the superior rectus and inferior oblique muscles, and therefore, it is likely they are both still activated after transposition. To act as a depressor, the transposed portion of the inferior oblique must be tethered at a position opposite the distal portion so that the muscle can contract between the two points. The distal inferior oblique is oriented in series with the NFVB after anterior transposition (Fig. 1), and contraction of the transposed muscle should induce tensile stress (that is, elongation) along the long axis of the NFVB. The NFVB, to serve as a functional tether to the posterior orbit, must be capable of tolerating this stress. In this study we tested the hypothesis that the NFVB has suitable mechanical properties to act as the ancillary origin of the inferior oblique muscle. Specifically, we determined stress–strain characteristics of the NFVB in vivo, in situ, and in vitro to assess its ability to tolerate the forces typical of normal eye movements. Our data indicate that when measured between 0% to 10% (0 to 2.1 mm) strain the NFVB was stiffer in vivo (11.8 MPa) than when measured either in situ (6.3 MPa) or in vitro in dynamic conditions (3 MPa; P < 0.05). These data suggest that the NFVB could act either alone or in concert with its adjacent orbital connections as an ancillary origin of the inferior oblique.

Our values for Young’s modulus of elasticity for the NFVB ranged from 0.7 to 12 MPa, considerably less than published stiffness values for tendons and ligaments (up to 1200 MPa for human tendon). However, the latter are musculoskeletal structures designed to withstand forces far in excess of those normally experienced by orbital tissues. Thus comparisons between orbital structures and tissues outside the eye may not be appropriate. For this reason we chose the SOT as a more relevant comparison for the NFVB. The tension required to maintain ocular fixation has been estimated to range from 79 to 393 mN (8 to 40 g), whereas the range of tensions required for torsional eye movements (in which the oblique muscles play a major role) is even less (4.9 mN/deg; 0.5 g/deg). A typical maximal torsional movement of 10° would require a 49 mN force. Based on a cross-sectional area of SOT of 0.034 cm² (Table 1), a 49-mN force would equal a stress of 0.014 MPa, whereas a 393-mN force would equal a stress of 0.12 MPa, requiring a strain of just slightly more than 10% (1.92
TABLE 2. Young’s Modulus (MPa) for the NFVB and SOT Determined From the Different Measurement Approaches

<table>
<thead>
<tr>
<th>Strain Range</th>
<th>NFVB</th>
<th>SOT</th>
</tr>
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<tbody>
<tr>
<td>5–10%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro (static; n = 9)</td>
<td>0.7 ± 0.3*</td>
<td>6.3 ± 0.4f</td>
</tr>
<tr>
<td>In vitro (dyn; n = 5)</td>
<td>3.0 ± 0.5†</td>
<td>18.2 ± 1.2f</td>
</tr>
<tr>
<td>In situ (static; n = 10)</td>
<td>6.3 ± 0.4f</td>
<td>2.6 ± 1.1</td>
</tr>
<tr>
<td>In vivo (static; n = 6)</td>
<td>11.8 ± 1.1§</td>
<td>3.5 ± 1.5</td>
</tr>
<tr>
<td>SOT</td>
<td>1.7 ± 0.7</td>
<td>2.6 ± 1.1</td>
</tr>
</tbody>
</table>

Values are mean ± SE.

* Values at each strain range are different from all others within the same method (P < 0.05).
† Dynamic (dyn) values are greater than respective static values (P < 0.05).
‡ Values in situ and in vivo are greater than those measured in vitro by static or dynamic methods at 5% to 10% strain (P < 0.05).
§ Value in vivo is greater than that in situ (P < 0.05).
|| The 20% to 25% values is greater than the 5% to 10% value within the same method (P < 0.05).

NFVB = neurofibrovascular bundle; SOT = superior oblique tendon.

mm), based on dynamic analysis (Fig. 4). Throughout a dynamic strain range of 5% to 10% for the SOT we determined a Young’s modulus of 1.7 MPa, similar to the maximum value of 2.5 MPa reported for the human orbicularis oculi muscle at 10% strain.19 By comparison, static stiffness of the NFVB was greater than that of the SOT in most strain ranges (P < 0.05, data not shown), whereas the dynamic stiffness of the NFVB was not significantly different from that of the SOT (Table 2).

Although the static relation indicated that the NFVB could tolerate strains of 30% without failing, we assessed the NFVB in dynamic conditions only up to a typical maximum in biomaterial strain of 10% (2.1 mm; Fig. 4).23>24 We believe the dynamic approach more accurately reflects the response of the NFVB because the eye is a highly mobile organ. We extended the dynamic curves for each sample of NFVB, using parabolic extrapolation (Fig. 5) and found that the dynamic stress–strain relations were stiffer at all intervals of strain compared with those in static in vitro measurements. As demonstrated (Fig. 5), the isolated NFVB in dynamic conditions could tolerate a modest stress (assuming a mean cross-sectional area for the NFVB of 0.0073 cm2; Table 1) up to 0.2 MPa (49 mN; 10 g) at 10% strain (2.1 mm); whereas at a maximum physiologic stress of 0.54 MPa (393 mN; 40 g), the corresponding strain would range between 15% and 20% (3.2 to 4.3 mm)—a level greater than the typical physiologic range of 4% to 10% for most tendons. We found that the Young’s moduli for the NFVB in situ and in vivo (both measured using intact orbits) were larger than that for the isolated NFVB. At 0.54 MPa stress, corresponding strain rises 8% (1.7 mm) in situ and 6% (1.3 mm) in vivo. In situ and in vivo, the NFVB demonstrates very clear linear stress–strain relations. The stiffness in vivo was greater than that in situ probably because the tissue was live and the subjects were young.31

In that stiffness both in vivo and in situ was greater than that in vitro, our data suggest that the ancillary origin of the inferior oblique muscle might originate from the NFVB alone or from the NFVB and its parallel orbital connections. The NFVB could act alone as an ancillary origin at forces up to 393 mN (0.54 MPa), assuming an in vivo resting strain between 15% to 20%. It is more likely that resting strain is less than 15%, and, therefore, additional orbital connections also contribute to the tethering effect. Based on the stress–strain relations in vivo or in situ, these two possibilities cannot be discriminated, although there is anatomic and radiographic evidence to suggest that in vivo the NFVB, and not Lockwood’s ligament, serves as the true functional tether.10,17,18 Recent anatomic
studies have demonstrated that fibrous tissue bands in parallel with the NFVB anteriorly may contribute to the tethering effect in vivo and in situ. Our findings demonstrate that the NFVB is mechanically capable of acting either alone or as a component of the ancillary origin of the inferior oblique muscle after anterior transposition.

Key Words
connective tissue, extraocular muscle, orbic, stiffness, strabismus

References