Increased Frequency of Activated Satellite Cells in Overacting Inferior Oblique Muscles from Humans

Rosâlia M. S. Antunes-Foschini,¹ Fernando S. Ramalho,² Leandra N. Z. Ramalho,³ and Harley E. A. Bicas¹

PURPOSE. Strabismus is an oculomotor disorder in which there is a misalignment of the visual axes of the eyes. Inferior oblique muscle (IOM) overaction is a common finding in comitant horizontal strabismus, but its origin is unclear. Recent studies have demonstrated that myogenic satellite cells (SCs) are still activated in adult extraocular muscles, with continuous myonuclear addition in normal uninjured muscles. The objective of this study was to determine whether there are differences in the processes of activation and proliferation of SCs in IOMs of patients with strabismus and IOM overaction and in patients with no history of strabismus.

METHODS. Cross sections of IOMs from strabismic and control groups were analyzed immunohistochemically for the presence of MyoD1 and myogenin, specific markers of activated SCs, and for c-Met, which is expressed in quiescent, activated, and proliferating SCs.

RESULTS. In overacting IOMs of 26 patients in the strabismic group and 10 patients in the control group, 28.8% and 3.0% of the myofibers, respectively, were associated with MyoD1-positive SC. The frequency of myogenin-positive SC was 30.8% in the strabismic group and 3.6% in the control group, and the frequency of presumptive SCs immunostained for c-Met was 33.6% in the strabismic group and 34.1% in the control group.

CONCLUSIONS. The presence of an increased number of activated SCs in overacting IOMs of the strabismic group in contrast to the frequency in the control group resembles the findings detected in developing, regenerating, or hypertrophic muscle tissue. High levels of MyoD1- and myogenin-positive SC in overacting IOMs support the hypothesis that these cells may be involved in alterations in IOM structure correlated with the overaction observed clinically. (Invest Ophthalmol Vis Sci. 2006;47:3360–3365) DOI:10.1167/iovs.05-0798

S trabismus is an oculomotor disorder in which there is a misalignment of the visual axes of the eyes, in at least one of the positions of gaze.¹ Its etiology has long been known to have a genetic component.² In some syndromes, such as Duane’s and Moebius syndromes, as well as in congenital fibrosis of the extraocular muscles (EOMs),³,⁴ some chromosomal loci have been identified. Amino acid substitutions due to point mutations have also been identified as the cause of congenital fibrosis of the EOMs.⁴

Comitant horizontal strabismus, such as the congenital or infantile esotropias and the exotropias, can be found in distinctly related members of the same family.⁵ In a significant percentage of cases of horizontal strabismus, oblique muscles are thought to be under- or overactive. In such cases, overaction of the inferior oblique muscles (IOMs) is assumed to be present when a “V” pattern (a relative divergence in upgaze when compared with downgaze) and hypertropia of the abducting eye are found.⁶ IOM overaction may be attributable to anomalies of scleral insertion of the medial rectus muscles, abnormal vestibular stimulation, weakness of the ipsilateral superior oblique muscles or the contralateral superior rectus muscles, or impulses spread along the third cranial nerve. Other possible causes are associated with structural anomalies of the face and the orbit, as well as ocular torsion.⁷ Until now, there is no evidence that IOM overaction is due to hypertrophy or hyperplasia⁸ or a pattern of overactive innervation of this muscle.

Although the reason for such primary IOM “overactions” is still unclear, when the condition is very strong, surgical treatment is applied by recessing the “affected” muscle, or by myectomy.

Mammalian EOMs are unique when compared with nonocular skeletal muscles. Myofibers of adult skeletal muscles continue to express several molecules that are normally downregulated in mature skeletal muscles and are associated only with skeletal muscle development or regeneration. These molecules include immature myosin heavy-chain isoforms,⁹ neural cell adhesion molecules,¹⁰ and muscle mitogens and growth factors.¹¹

Skeletal muscle is postmitotic in adults; however, a population of pluripotent mononucleated cells, which are situated between the outer sarcolemma and the basal lamina of the myofibers,¹² are responsible for muscle repair and regeneration. In the unperturbed state, satellite cells are normally quiescent, becoming activated when the muscle suffers regeneration, development, or training.¹³ However, in the unperturbed EOMs of adult humans there is a small percentage of activated satellite cells,¹⁴ positive for a myogenic lineage-specific marker, MyoD. MyoD is expressed in both activated satellite cells and myoblasts,¹⁴,¹⁵ but not in quiescent satellite cells,¹⁶ myotubes, or mature myofibers. Furthermore, a continuous process of myonuclear addition to normal uninjured myofibers occurs in EOMs.¹⁷ Myogenin is another myogenic regulatory factor that is observed in activated satellite cells from skeletal muscles¹⁵ and EOMs.¹¹ In culture, it appears after MyoD1 expression and before developmental myosin.¹⁸ c-Met, the receptor for hepatocyte growth factor (HGF), a multifunctional cytokine expressed on skeletal muscle that activates satellite cells through interaction with its receptor, is another effective molecular marker for quiescent, activated or proliferating satellite cells. Cornelison and Wold¹⁶ detected it beneath the basal lamina of presumptive satellite cells in intact muscle and also observed in satellite cell cultures that c-Met mRNA and protein are expressed by all myofiber-associated satellite

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Supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

Submitted for publication June 23, 2005; revised October 25, 2005, and January 12, and February 23, 2006; accepted June 19, 2006.

Disclosure: R.M.S. Antunes-Foschini, None; F.S. Ramalho, None; L.N.Z. Ramalho, None; H.E.A. Bicas, None

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cells. c-Met was not detected in fibroblasts or other mononucleated cells from healthy muscle explants. Barani et al. described a significant increase in the expression of c-met in proliferating myoblasts. McLoon et al. observed that 17% of the myofibers had associated c-Met-positive satellite cells in EOM from rabbits.

The above information can provide the basis for many hypotheses that may explain the oculomotor disorders present in strabismus. Disorders in the ability of differentiation and proliferation of satellite cells or differences in the number of satellite cells may be correlated to heterogeneity in EOM structure. Different abilities in remodeling EOMs may lead to ocular imbalance and then to strabismus. In the present investigation, we studied the activated satellite cells by quantifying the lineage-specific markers Myo-D1 and myogenin and the total number of satellite cells, by quantifying presumptive satellite cells immunolabeled for the anti-c-Met antibody in IOMs of patients with strabismus and overaction of the IOMs and from cornea donors without a previous history of strabismus. We also looked for myofiber hypertrophy by measuring the fibers’ cross-sectional areas.

**METHODS**

The study included 26 patients with a diagnosis of strabismus (14 men and 12 women), who attended the University Hospital (Faculty of Medicine of Ribeirão Preto, University of São Paulo, Brazil). The age of the patients ranged from 3 to 56 years (mean, 17.8 ± 13.3). All patients presented with strabismus secondary to high overaction of the IOM, associated or not with horizontal deviations, and were treated surgically, under general anesthesia, by myectomy of the IOM (strabismic associated or not with horizontal deviations, and were treated surgically). Patients with strabismus secondary to high overaction of the IOMs and from cornea donors were selected without a previous history of strabismus. Patients’ data are shown in Table 1. Ten IOM samples were obtained from cornea donors (mean, 25.7 ± 10.3 years), whose causes of death were traumas or infectious diseases (control group). None had a history of strabismus or neuromuscular diseases (Table 2). The study protocol conformed to the ethical guidelines of the 1995 Helsinki Declaration (as revised in Edinburgh 2000) and was approved by the local Ethics Committee.

All muscle specimens had been routinely fixed in 4% neutral formalin and embedded in paraffin blocks. The mean time from death to the time of fixation was 9.2 ± 6.3 hours in the control group (Table 2), whereas specimens obtained from myectomies (strabismic group) were fixed immediately. Four-micrometer-thick cross sections mounted on poly-l-lysine–coated slides were deparaffinized, rehydrated, immersed in 10 mM citrate buffer (pH 6.0), and submitted to heat-induced epitope retrieval using a vapor lock for 45 minutes. The slides were briefly rinsed with phosphate-buffered saline (PBS) and immersed in 3% hydrogen peroxide for 5 minutes to block endogenous peroxidase. Nonspecific protein binding was blocked with normal serum (Universal Quick Kit; Novocastra, Newcastle, UK) for 10 minutes. Next, the slides were incubated with the streptavidin-biotin complex reagent (Universal Quick Kit; Novocastra) for 5 minutes and developed with 3,3-diaminobenzidine tetrahydrochloride (Liquid DAB Substrate Kit; Novocastra) for 5 minutes. The sections were counterstained with Mayer hematoxylin and mounted (Permount; Fischer Scientific, Pittsburgh, PA). Rhabdomyosarcoma biopsy specimens were analyzed (R, right; L, left; percentage of satellite cells positive for c-Met, MyoD1, and myogenin; IM, insufficient material).

### Table 1. Strabismic Group: Clinical and Immunohistochemical Findings

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<th>AS (y)</th>
<th>AS-AD (y)</th>
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<th>Horizontal Deviation</th>
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<th>IOM Analyzed</th>
<th>c-Met (%)</th>
<th>MyoD1 (%)</th>
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**n = 26.**

AD, age (y) strabismus presumably began; AS, age (y) at the time of surgery; AS-AD, interval between them; associated horizontal deviation: ET, esotropia; XT, exotropia; alt, alternant; RE, right eye; LE, left eye; presence of amblyopia or low visual acuity (VA) and their causes; CC, congenital cataract; Ch, chorioretinitis; HM, high myopia; IOM analyzed (R, right; L, left); percentage of satellite cells positive for c-Met, MyoD1, and myogenin; IM, insufficient material.
used as the positive control, and negative controls were prepared by omission of the primary antibodies.

The preparations were evaluated blindly in 20 high-power (×100) fields chosen at random by an experienced pathologist. The results are reported as the percentage of MyoD1-positive nuclei and myogenin-positive nuclei (positive nuclei/100 counted myofibers), and as the c-Met labeling index, representing the percentage of presumptive satellite cells in relation to the myofibers counted. Cross sections routinely stained with hematoxylin-eosin were also submitted to measurement of myofiber areas, in circular cross-sectional areas. Oblique (or oval) sections, in which it was possible to see microstriations, were not considered.

All data are reported as mean ± SD. Statistical comparisons of the groups were performed by the nonparametric Mann-Whitney test. P < 0.05 was considered to be statistically significant.

**RESULTS**

A summary of the clinical data, as well as the percentage of satellite cells immunostained for MyoD1, myogenin, and c-Met in overacting IOM from patients in the strabismic group is shown in Table 1. Table 2 shows data from the control group.

The mean cross-sectional areas of myofibers were 733 ± 256 µm² in the strabismic group and 697 ± 172 µm² in the control group (P > 0.05; Fig. 2).

The case of patient 1 from the control group, whose muscle was obtained in a manner similar to the specimens from the strabismic group, was interesting, in that the muscle had the same percentages of MyoD1-, myogenin-, and c-Met-positive satellite cells as the other donors in the control group, from whom specimens were obtained some hours after death.

A subgroup of eight patients, seven of them from the strabismic group (see data in Table 1), had bilateral IOM overaction and had been submitted to bilateral myectomy for surgical correction of their deviations. Both IOMs from each patient had high frequencies of MyoD1 immunolabeling (29.1% ± 7.8% for the right IOM and 30.2% ± 6.6% for the left IOM). The mean cross-sectional areas of myofibers were 733 ± 256 µm² in the strabismic group and 697 ± 172 µm² in the control group (P > 0.05; Fig. 2).

**DISCUSSION**

The strabismic group, with 26 patients, was a highly heterogeneous group, with a wide variability of ages at the time of surgery (3–56 years) and with horizontal deviations varying from exotropia to esotropia, and absent in 3 subjects. Although almost two thirds of them (17 patients) did not have amblyopia or low visual acuity due to other causes, there were also patients with amblyopia (6 patients) or low visual acuity due to other causes (high myopia, chorioretinitis, and congenital cataract: 4 patients). Most of them (23 patients) knew the age when deviation had started, with an early onset, before 2 years of age, being reported by 19. The age at the onset of strabismus ranged from 0 to 30 years, and the interval between onset and surgery also varied widely from 3 to 35 years. Some of the patients (patients 1, 2, 3, 4, 7, 9, and 13 [Table 1], in addition to another one), had symmetric bilateral overacting IOM, justifying bilateral myectomy, whereas the others had only one overacting IOM, or bilateral asymmetric IOM overactions that did not justify bilateral myectomy. Although the strabismic group was heterogeneous in many clinical aspects, all the

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**Table 2. Control Group: Clinical and Immunohistochemical Findings**

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<tr>
<th>Control</th>
<th>Age</th>
<th>Cause of Death</th>
<th>Sex</th>
<th>Interval (h)</th>
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<th>MyoD1 (%)</th>
<th>Myogenin (%)</th>
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**FIGURE 1.** Expression of MyoD1 and myogenin in cross sections of inferior oblique muscles. Arrows: satellite cell nuclei (reddish-brown nuclei) immunostained for MyoD1 (A) and myogenin (B) in overacting IOM from the strabismic group and one satellite cell nucleus positive for MyoD1 in IOM from the control group (C). Original magnification, ×100.
patients had misalignment of the visual axes, at least in one position of gaze, with a great overaction of the IOM, justifying myectomy as the surgical treatment of the IOM overaction.

Besides IOM overaction, some other etiologies may explain overelevation in adduction, such as upshoot, which occurs in Duane’s syndrome, attributed to the co-contraction of the medial and lateral recti muscles, contralateral superior rectus muscle palsy, or pseudoveraction of the inferior (and the superior) oblique muscles in exotropic patients. However, the patients in the strabismic group did not have other clinical findings that would explain these alternative etiologies. In exotropic patients, pseudoveraction of both superior and inferior oblique muscles is frequent and usually associated with “X” patterns. Eleven of 26 patients from the strabismic group were exotropic, but none of them had associated “X” patterns. Seven of 11 had a “V” pattern of >15°; and 4 also had a “V” pattern smaller than 15°.1.1

The specimens from the control group were obtained from cornea donors whose causes of death were trauma, infectious diseases, neoplasia, and pulmonary edema. None of the donors had a history of strabismus or neuromuscular diseases (Table 2).

Immunohistochemistry showed a wide variability in the frequency of MyoD1- and myogenin-positive satellite cells in the strabismic group and in the control group (P < 0.0001). The percentage of satellite cells immunostained for MyoD1 and myogenin was approximately 10 times higher in the strabismic group than in the control group. The age of the patients at the time of surgery varied between the strabismic and control groups. Only 2 patients of 10 were 12 years old or younger in the control group, whereas 10 of 26 in the strabismic group were less than 12 years old. Although the ages of the patients from the strabismic and control groups were not uniform, the pattern of MyoD1 and myogenin positivity did not appear to be consistently associated with age, since the results were homogeneous in two groups.

MyoD and myogenin belong to a family of helix-loop-helix proteins that control myogenic differentiation. They are excellent markers for activated satellite cells15,16,21 and myoblasts. Activated satellite cells are usually observed in high levels in developing (growing) and regenerating muscles. In these situations, they are ready to differentiate and fuse with preexisting myofibers or to generate new myofibers.13,22 In contrast, quiescent SC, myotubes, or mature myofibers from adult skeletal muscle do not express MyoD1.15 Hypertrophy is also associated with an increase in frequency of satellite cells in skeletal muscle, as well as in the number of myonuclei and myofiber areas.13,23,24

As stated earlier, muscle regeneration after mechanical or chemical trauma is accompanied by an increase in the number of activated satellite cells. Indeed, many muscle-regeneration models are used to study the process of activation of satellite cells.25 In the present study, none of the patients in the strabismic group had a history of acute muscular or orbital trauma before surgery, which may explain the high frequency of activated satellite cells in this group. Surgical trauma, however, could be responsible for the differences found, since IOMs from the control group were obtained from cadavers. IOM myectomy, however, is a rapid procedure, lasting approximately 15 to 30 minutes from the opening of the conjunctiva, through the myectomy, and closure of the surgical wound, and the IOMs were immediately immersed in buffered formalin. Furthermore, Koishi et al.26 observed no MyoD-positive satellite cells immediately, with the first MyoD-positive satellite cells appearing 1 day after trauma near the damaged area. Grounds et al.15 detected increased sequences of MyoD1 mRNA in mononuclear cells from skeletal muscle as early as 6 hours after injury, peaking between 24 and 48 hours. Rantanen et al.27 reported that the first MyoD1-positive myoblasts after contusion injury to the gastrocnemius or after toxic injury to the soleus muscle were seen after 12 hours. McLoon et al.28 found that maximum activation of satellite cells, as defined by MyoD detection, occurred 2 and 3 days after bupivacaine injection to the orbicularis oculi muscle. Other investigators have confirmed these findings.29 In the present study, control subject 1 of the control group had his IOM removed in a procedure similar to that used in the strabismic group. He was the donor of multiple organs, and his organs, as well as his ocular globes, were excised under general anesthesia. The frequency of his IOM MyoD1- and myogenin-positive satellite cells was similar to that observed in other IOM from the control group. Thus, the hypothesis of an influence of surgical trauma leading to high frequencies of MyoD1-positive satellite cells in the strabismic group is unlikely.

A second condition in which there is an increase in the number of MyoD1-positive satellite cells occurs in immature myofibers, which are still growing, such as myofibers of neona-
tes.26 IOM from the strabismic group resembles immature tissues, as they express 10 times more MyoD1- and myogenin-positive satellite cells than IOM from control group. It is important to remember that 19 of 26 patients in the strabismic group had a history of ocular deviation starting early in life, before 2 years of age, suggesting early muscular alterations of long duration. Even deviations of 15 years or more (patients 13–19, 23; Table 1) maintained the structural alterations found in younger patients in the strabismic group. There were also patients with deviations that appeared late (patients 11, 21, 24, 25, Table 1). However, none of them reported any occurrence that might really define the exact time when deviation appeared, such as cranioencephalic trauma that could lead to ipsilateral superior oblique muscle paresis or palsy, with later overaction of the IOMs. It is possible that, in these patients, deviation occurred before the time reported by them. A possible explanation is that these patients, probably with early deviations, were asymptomatic, or barely symptomatic for long periods, and compensated for their deviations (vertical phorias, for example) by more efficient fusional mechanisms. Thus, although the strabismic group was clinically heterogeneous, it
probably consisted of patients with the same kind of affliction (i.e., overacting IOM), with a high number of activated satellite cells leading to different ocular imbalances, depending on the heterogeneous capability to compensate for these deviations, besides other factors, such as good visual acuity in both eyes or not or presence of associated horizontal deviations. Different abilities of activation and differentiation of satellite cells from strabismic and control groups might explain these data. Barani et al.19 observed that the proliferation of a population of satellite cells is asynchronous, being possibly heterogeneous. Some cells can act as myogenic precursor cells, proliferating, whereas others are ready for differentiation and later fusion with preexisting myofibers.

A correlation between hypertrophy, increases in the number of satellite cells, and increases in muscle strength has also been demonstrated by many authors. Kadi et al.35 reported it in high-level weight-lifting athletes who had used high doses of anabolic steroids for several years. Kadi and Thornell24 observed it in female trapezius muscles after strength training. Testosterone-induced muscle fiber hypertrophy was also associated with an increase in the number of satellite cells, a proportional increase in the number of myonuclei, and changes in satellite cell ultrastructure.29 Vierck et al.30 also showed that creatine, an ergogenic compound in the monohydrate form, known to increase muscular mass and strength in athletes, induced differentiation but not proliferation of muscular satellite cells in vitro. Based on these data, IOM hypertrophy was investigated in the two groups by the measurement of myofiber areas. Although satellite cells immunostained for MyoD1 and myogenin were more frequent in the overacting IOM group (strabismic group), we did not find a significant difference in the increase in mean cross-sectional areas between the strabismic group (733 ± 256 μm²) and the control group (697 ± 172 μm²). Indeed, although there is the impression that overacting IOMs are found to be hypertrophic during surgery, this has not been confirmed by magnetic resonance imaging.8 However, recent work has demonstrated that different types of skeletal muscles present different modes of hypertrophy.31 Muscles with a single endplate band respond to hypertrophy by increasing myofiber cross-sectional areas, whereas muscles with multiple endplate bands do not respond in this way but rather increase individual myofiber length. The cross-sectional area heterogeneity of the strabismic group is an interesting finding and may be correlated to this mode of hypertrophy. So, although hypertrophy was not confirmed by an increase in mean cross-sectional areas, it cannot be discarded.

McLoon and Christiansen32 studied the short-term effects of insulin-like growth factor (IGF)-II (IGFs are growth factors that induce proliferation and differentiation of satellite cells) on the morphometry of EOMs from the rabbit superior rectus. They found increased force generation associated with an increase in the heterogeneity of myofiber cross-sectional areas, with increases in both small and very large myofibers and with no significant change in the mean cross-sectional areas of myofibers compared with the control. McLoon (personal communication, 2005) also found increases in the frequency of MyoD1-positive satellite cells in this group, showing that increases in muscular strength are associated with increases in MyoD1-positive satellite cells. Based on these data, we could hypothesize that the IOMs are overactive because of increases in their strength, directly associated with increases in the frequency of MyoD1-positive satellite cells.

Although patterns of innervation of IOMs were not specifically studied, their influence may be related to the differences in the number of satellite cells found in control and strabismic groups. Satellite cell density is higher in the proximity of myoneural junctions,9,13 suggesting that neurotrophic factors are probably important in their homeostasis. In contrast, there is a decrease in the number of satellite cells and an increase in their susceptibility to apoptotic cell death55 when a muscle undergoes long-term denervation.54 The current study showed that overacting IOM have higher levels of MyoD1- and myogenin-positive satellite cells that may be responding to increased contraction or increased force generation by increasing the number of MyoD1- and myogenin-positive satellite cells (McLoon LK, personal communication, 2005),23,24,29,50 imposed on them by the oculomotor nerve.

MyoD1 and myogenin may be proteins with altered expression in oculomotor disorders. Work with visual deprivation models that mimic what occurs in untreated strabismus or amblyopia detected alterations in many EOM-related proteins, such as myosin heavy-chain isoforms35 and others,36 and confirmed the hypothesis that visual input to the oculomotor system during development modulates EOM-specific proteins.

Another possibility is that satellite cells of patients with strabismus may become activated and ready to differentiate, expressing MyoD1 and myogenin, but then becoming apoptotic.37 That is, although they express MyoD1 and myogenin, there is no later expression of other terminal markers such as developmental myosin.18 Immunolabeling for c-Met did not detect differences between the control group (34.1% ± 1.8%) and the strabismic group (33.6% ± 2.5%). These values show a considerable quantity of presumptive satellite cells per total number of myofibers analyzed. McLoon et al.28 observed in EOMs from rabbits (rectus muscles) that approximately 17% of myofibers had associated c-Met-positive satellite cells, a smaller percentage than we found. However, the present study was conducted on IOMs from humans.

This article may help other investigators to elucidate better the physiopathology of some of the various forms of comitant strabismus.

Acknowledgments

The authors thank Auristella de Mello Martins and Alexandre da Silva Roque for technical assistance.

References

9. McLoon LK, Rios I, Wirtshaifer JD. Complex three-dimensional patterns of myosin isoform expression: differences between and


