

Increasing Muscle Strength as a Treatment for Strabismus: Sustained Release of Insulin-like Growth Factor-1 in Rabbit Extraocular Muscle

Linda K. McLoon, PhD,^{a,b} Brian C. Anderson, BS,^a and Stephen P. Christiansen, MD^{a,c}

PURPOSE

Currently, no drug treatment is available for strengthening underacting extraocular muscles (EOM) in strabismus. We showed previously that single injections of insulin-like growth factor (IGF-1) result in significant but short-term increases in muscle force generation. This study examined the effects of sustained release of IGF-1 on force generation in rabbit superior rectus muscles.

METHODS

In adult rabbits, slow-release pellets containing IGF-1 were implanted on the global side of one superior rectus muscle. After 1 week, or 1, 2, 3, or 6 months, treated and control muscles were examined for force generation using an in vitro physiology apparatus. All muscles were prepared for histology and mean myofiber cross-sectional areas were determined.

RESULTS

One and 3 months after pellet implantation, treated muscles generated significantly greater force than contralateral control muscles, whereas at 2 months, no significant difference was found. Force per cross-sectional area (mN/cm²) at 3 months also increased significantly in the treated muscles. Mean muscle cross-sectional area increased significantly after 1, 2, and 3 months of sustained exposure to IGF-1 compared with controls. After an additional 3 months without IGF-1 exposure, mean cross-sectional areas were significantly greater than controls but significantly reduced compared with areas at 1, 2, and 3 months.

CONCLUSIONS

IGF-1 appears to be highly effective in increasing muscle force generation. Because slow release of IGF-1 results in sustained increases in EOM force generation, it may be a potentially useful alternative to surgical resection procedures because it avoids many of the potential long-term biomechanical hazards of resection surgery. (J AAPOS 2006;10:424-429)

The surgical treatment of most forms of strabismus entails either recession to weaken an overacting muscle or resection to strengthen an underacting muscle. For patients with large-angle horizontal deviations of greater than 30^Δ, repeat surgery is needed to achieve or maintain satisfactory alignment in approximately 25% to 69% of strabismic patients.^{1,2} This may be the result, in

part, of biomechanical changes induced by the surgery itself that adversely affect rotations or extraocular muscle (EOM) function. Surgical alterations in the muscle insertion, changes in the arc of rotation, scarring, long-term changes in muscle resting length, changes in twitch tension, and disruption of normal EOM-pulley interactions may all have short and long-term effects on EOM function and, secondarily, on alignment.³

Normal binocular alignment is a complex balance of motor tone and orbital elastic factors, both passive and active, and is principally driven by motor and sensory fusional mechanisms. Changes in motor tone or strength then result in changes in the rotational position of the globe. An attractive alternative to incisional surgery that has not been adequately investigated is pharmacologic modulation of EOM force generation. By weakening or strengthening an EOM with an injectable agent, globe rotational changes can be accomplished without altering normal EOM relationships with the globe or with surrounding orbital tissues. Botulinum toxin A, first introduced in the 1970s, was the first such agent,⁴ and it has been used with measured success for the treatment of some types of childhood and adult strabismus.⁵ Its use has been

Author affiliations: ^aDepartment of Ophthalmology, University of Minnesota, Minneapolis, Minnesota; ^bNeuroscience, University of Minnesota, Minneapolis, Minnesota; ^cPediatrics, University of Minnesota, Minneapolis, Minnesota

Presented at the 32nd Annual Meeting of the American Association for Pediatric Ophthalmology and Strabismus, Keystone, Colorado, March 15-19, 2006.

Supported by EY15313 and EY11375 from the National Eye Institute, the Minnesota Medical Foundation, the Minnesota Lions and Lionesses Research to Prevent Blindness Lew Wasserman Mid-Career Development Award (LKM) and an unrestricted grant to the Department of Ophthalmology from Research to Prevent Blindness Inc.

Submitted April 10, 2006.

Revision accepted June 25, 2006.

Reprint requests: Linda McLoon, PhD, Department of Ophthalmology, University of Minnesota, Room 374 LRB, 2001 6th Street SE, Minneapolis MN 55455 (email: mcloo001@umn.edu).

Copyright © 2006 by the American Association for Pediatric Ophthalmology and Strabismus.

1091-8531/2006/\$35.00 + 0

doi:10.1016/j.jaapos.2006.06.011

limited, however, by the fact that it can only weaken muscle and by the relatively short duration of its paralytic effect. It can, however, result in permanent realignment in both adults and children. Critical to the success of drug treatment for strabismus are combinations of agents that weaken or strengthen EOM and that have sufficient duration of action that central nervous system adaptation, restoration of fusion, and long-term adjustments in EOM length occur to yield satisfactory alignment that persists. However, permanent derangements of EOM force generation are not ideal as these could lead to consecutive strabismus.

We have shown, in earlier studies, that direct injection of ricin-mAb35 yields long-term but reversible decreases in EOM force generation in rabbits.⁶ It seems reasonable that strengthening an EOM could be equally valuable in a clinical setting. Our previous work demonstrated that injection of either insulin-like growth factor (IGF)-1 or -2 into adult rabbit superior rectus (SR) muscles results in significant increases in muscle mass and force generation.^{7,8} These effects, however, are short-lived, and the EOM force generation returns to control values by 14 days. In another study by other investigators, retrobulbar injection of a cocktail of growth factors resulted in a short-term increase in muscle force in chick extraocular muscles.⁹ These studies demonstrate that a pharmacological approach to muscle strengthening is reasonable and possible. If increases in force generation can be sustained, the combination of long-acting weakening and strengthening agents may allow treatment of strabismus without the biomechanical hazards of traditional surgery.

There are a number of methods described in the literature for sustained release of growth factors such as IGF to deliver a therapeutic dose to skeletal muscle. Most of these methods, however, are not clinically relevant to the development of growth factor treatments for strabismus; they use transgenic mice that overexpress IGF-1 or viral vector upregulation of IGF expression.¹⁰⁻¹² The use of slow-release, implantable pellets that we report here allows for sustained release of low levels of IGF-1 in a targeted muscle and is an approach that has potential clinical application. The purpose of this report is to describe the effects of sustained-release IGF-1 on motor force generation and mean myofiber cross-sectional area at 1, 2, 3, and 6 months after implantation.

Materials and Methods

Adult New Zealand white rabbits were obtained from Bakkom Farms (Red Wing, MN) and housed with Research Animal Resources. All studies were approved by the Institutional Animal Care and Use Committee at the University of Minnesota in compliance with the NIH and ARVO guidelines for the use of animals in research.

Rabbits were anesthetized by an intramuscular injection of ketamine, 10 mg/kg, and xylazine, 2 mg/kg. Proparacaine HCl drops were placed in the conjunctival cul-de-sac to reduce blink-

ing during pellet implantation. Using sterile conditions, the conjunctiva was opened to allow access to the SR muscle. A sustained-release pellet containing IGF-I was positioned on the global side of one SR, randomized preoperatively. The pellets contained 180 μg of IGF-1 (PeproTech Inc., Rocky Hill, NJ), and were calibrated to release 2 μg IGF-1/day for a period of up to 90 days (Innovative Research of America, Sarasota, FL). The contralateral SR was implanted in an identical manner with a placebo pellet. The rabbits were allowed to survive for 1 week, and 1, 2, 3, or 6 months. A total of 6-8 rabbits were used for each experimental endpoint.

At each postimplantation interval, the rabbits were deeply anesthetized with ketamine and xylazine. The animals were euthanized by thoracotomy followed by exsanguination. Both SR muscles were removed completely from their origin at the orbital apex to their scleral insertion. The muscles were placed immediately into oxygenated Ringers solution at 30°C and, based on previous *in situ* measurements, they were carefully pinned to their normal *in situ* length. A 4-0 silk loop was tied to the end of each muscle. The upper loop was attached to a lever arm connected to a force transducer, and the muscles were suspended in *in vitro* incubation chambers and continually bathed in oxygenated Ringers at 30°C for the duration of the experiment. Generated force in grams was recorded using the 1205 Intact Muscle Test System and Dynamic Muscle Control software (Aurora Scientific, Aurora, Ontario, Canada). To account for force generation caused by differences in muscle size, stress was calculated as force divided by muscle cross sectional area. Muscle length and mass were obtained, and muscle cross sectional area was determined by dividing muscle mass (g) by the product of muscle length (cm) times a muscle density of 1.056 g/cm³. This yielded stress in g/cm², which was converted to mN/cm², eliminating muscle size as a factor when comparing force generation between the injected and noninjected muscles. Previous studies have demonstrated that there is a 5% variation between the right and left sides of normal extraocular muscles in the same animal, and this system can detect increases above that value only.⁸ Despite the fact that in every case, the muscle exposed to IGF-1 showed elevated force generation compared with the contralateral untreated control muscle in the same animal, there is sufficient interanimal variability of force generation in the control muscles that 6-8 experimental animals are required at each postinjection interval to show a significant treatment effect.

Physiological force measurements were performed on both the control and IGF-I-treated muscles by methods previously described.^{7,8} In brief, isometric length-tension curves were determined by stimulating each muscle at supramaximal intensity (500 mA, 0.5 ms) while varying the preload (resting length) over a range of 0.5 g-10 g. All physiology was performed with supramaximal stimulus intensities at optimal preload. Muscles were stimulated at frequencies of 10, 20, 40, 100, 150, and 200 Hz at a train duration of 500 ms with a 2-minute rest between each stimulation. After 2 minutes' rest, the muscles were subjected to a fatigue protocol where a tetanic stimulus was delivered every 2 seconds, consisting of a 1-second train at 150 Hz. The muscles were stimulated for 600 seconds or until there was a 50% reduction in generated muscle force. Data from treated and control

muscles were pooled at each postinjection interval and compared with the paired *t*-test. A *p*-value ≤ 0.05 was considered statistically significant.

The SR muscles were removed from the *in vitro* apparatus, embedded in tragacanth gum, and frozen in methylbutane chilled to a slurry on liquid nitrogen. The muscles were serially sectioned at 12 μm and immunostained for visualization of the fast myosin heavy chain isoform (MyHC). The sections were rinsed in phosphate-buffered saline, followed by incubation with the antibody to fast MyHC (1:40 Novocastra, Vector Labs, Burlingame, CA), followed by incubation using the Vectastain peroxidase ABC kit (Vector Labs). The peroxidase was developed using 3,3'-diaminobenzidine (DAB) and hydrogen peroxide with heavy metal intensification. Cross-sectional areas were determined by manual tracing of myofibers using the Bioquant Nova Prime morphometry software. For all measurements, counts and area measurements were made in a minimum of 6 random fields within both the orbital and global layers of both the IGF-1 treated and control rectus muscles from 6 rabbits each at all 4 postimplantation survival times. Data from each set of treated and control muscles were pooled for each postimplantation interval and compared with the paired *t*-test aided by the Prism and Statmate software (Graphpad, San Diego, CA). An *F*-test was used to verify that the variances were not significantly different. A *p*-value ≤ 0.05 was considered statistically significant.

Results

To verify that the effects on generated tension resulting from sustained-release delivery of IGF-1 from the implanted pellet at 2 $\mu\text{g}/\text{day}$ were similar at one week to direct injection, we compared data with our earlier published studies of direct injection of 5 μg IGF.⁸ Both groups showed significantly increased force generation compared with control SR muscles when examined as force in grams (Figure 1a) or when calculated as force per muscle cross-sectional area (Figure 1b).

After 1 and 3 months of sustained release at 2 $\mu\text{g}/\text{day}$, muscle force in grams was significantly increased compared with control values (Figure 2 and Table 1). There was a decrease in the force generated in muscles treated for 2 months compared with 1 or 3 months (Figure 2 and Table 1). When force generation was normalized to cross-sectional area and recalculated as mN/cm^2 , force remained increased over controls at 1 and 3 months but not at 2 months (Figure 3 and Table 2). Moreover, the decrease in force, when normalized to account for cross-sectional area, suggests that the muscle mass had significantly increased. By 6 months after pellet implantation, which is 3 months after the delivery period of the pellets, there was no statistical difference in the forces produced when the right and left SR muscles were compared.

Interestingly, several twitch contraction characteristics were altered after 3 months of sustained release of IGF-1 (Table 3). Total contraction time is longer, time to peak force is shorter, and the derivative of the maximal force generated over time slope is greater, suggesting a more

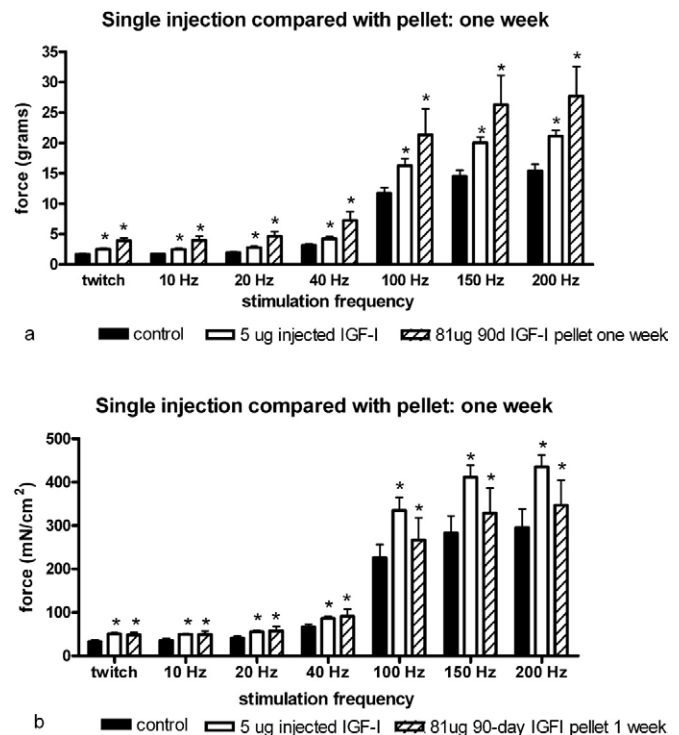


FIG 1. Comparison of force generation 1 week after pellet implantation dosing at 2 $\mu\text{g}/\text{day}$ (hatched), a single 5 μg injection of IGF-1 (white) compared with control values (black). (a) Force in grams. (b) Force in mN/cm^2 . *Significant difference from control animals.

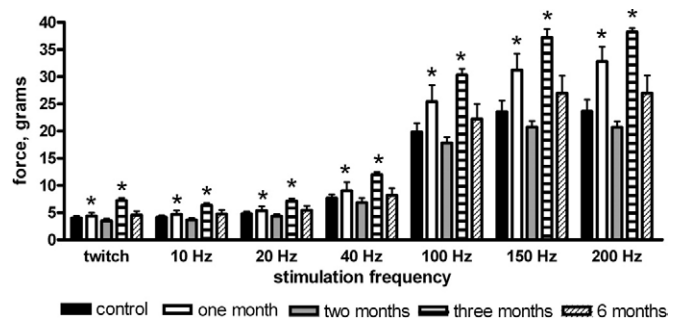


FIG 2. Force generation in grams after sustained release of 2 $\mu\text{g}/\text{day}$ for 1, 2, 3, or 6 months. *Significant difference from control animals.

forceful contraction. The time it takes for the IGF-1 treated muscles to reach half relaxation time is slightly longer compared with control, suggesting that it takes the treated muscles longer to relax from its higher stress or force output. By 6 months, the twitch characteristics were similar to the saline-treated control muscles.

No change in the fatigue properties were observed in the animals subjected to sustained-release IGF-1 (data not shown), as was seen after single injections of either IGF-1 or IGF-II.^{7,8}

Mean cross-sectional areas were determined for the SR muscles that had been exposed to either IGF-1 or the placebo for 1, 2, and 3 months, as well as for the muscles

Table 1. Force measurements in grams

Stimulation frequency	Control	One month	Two months	Three months	Six months
Twitch	4.0 ± 0.42	4.5 ± 0.59	3.5 ± 0.37	7.3 ± 0.44	4.5 ± 0.76
10 Hz	4.14 ± 0.38	4.74 ± 0.6	3.7 ± 0.34	6.4 ± 0.41	4.78 ± 0.75
20 Hz	4.8 ± 0.42	5.4 ± 0.6	4.34 ± 0.41	7.2 ± 0.4	5.4 ± 1.3
40 Hz	7.69 ± 0.7	9.1 ± 1.0	6.9 ± 0.84	12.0 ± 0.4	8.23 ± 1.2
100 Hz	19.82 ± 1.6	25.4 ± 2.05	17.8 ± 1.1	30.4 ± 1.1	22.22 ± 2.8
150 Hz	23.54 ± 2.0	31.3 ± 3.0	20.74 ± 1.2	37.2 ± 1.5	27.0 ± 3.18
200 Hz	23.6 ± 2.1	32.9 ± 2.6	20.7 ± 1.1	38.3 ± 0.6	27.0 ± 3.2

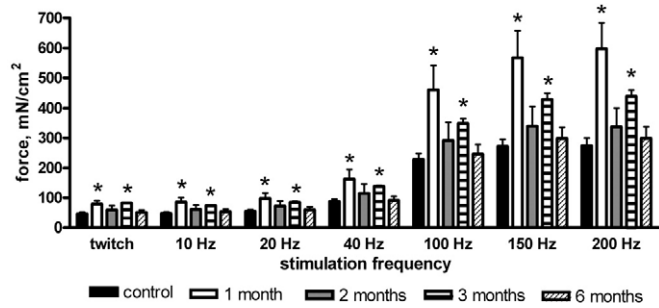


FIG 3. Force generation in mN/cm² after sustained release of 2 μ g/day for 1, 2, 3, or 6 months. *Significant difference from control animals.

that were left an additional 3 months after the end of the sustained IGF-1 release. After 1, 2 or 3 months of sustained exposure to IGF-1, mean muscle cross-sectional area was significantly increased compared with the contralateral control muscles (Figure 4). There was no overt evidence for increases in connective tissue or vasculature, although this was not quantified. By 6 months after implantation of the 90-day release pellets, the mean cross-sectional area had decreased significantly compared with that seen at 3 months. In all other ways, the tissue looked normal. The mean cross-sectional area at 6 months did not return completely to normal levels and was significantly greater than control values.

Discussion

Sustained-release of IGF-1 in rabbit SR muscle results in a prolonged increase in muscle force when assessed as either total grams of force generation or when normalized to account for muscle mass and expressed in mN/cm². In addition, the sustained release of IGF-1 results in increased muscle cross-sectional area at 1, 2, and 3 months after treatment, with increasing mean cross-sectional areas as duration of exposure increases. The effect of duration of treatment directly correlates with the duration of IGF-1 release from the implanted pellet. These results suggest that this technique may be a viable means of increasing and maintaining muscle force in adult extraocular muscles. While mean area had not returned to normal values by 6 months, the trend suggests that the mean fiber size would eventually return to control values.

Other than force generation, the EOM twitch characteristics of the treated muscles were remarkably normal.

Although the IGF-1-treated muscles generated more force, the overall twitch properties did not change dramatically compared with control muscles. This result might be caused by the known heterogeneity in MyHC isoforms present in individual EOM myofibers that influence overall muscle contractile properties. Therefore, properties such as stress and maximum dF/dt may increase, but the temporal properties tend to be stable.

Elevation of IGF-1 levels in nonocular skeletal muscle is effective in increasing both muscle cross-sectional area and muscle force generation in a wide array of muscle injury or muscle degenerative conditions. When viral vectors or transgenic mice are used to overexpress IGF-1, muscle cross-sectional area and muscle force generation were significantly increased in the mdx mouse, a model of muscular dystrophy.^{10,12,13} Increased muscle expression of IGF-1 increased muscle mass and force in both aging animals^{11,14} and in animal models and humans with cachexia.^{15,16} This finding supports the use of IGF-1 to safely increase muscle mass and muscle force generation in the treatment of a wide variety of muscle degenerative conditions. However, high-dose systemic administration has been associated with more side effects than therapeutic relief.¹⁷ The use of sustained release of IGF-1 through implantable pellets resulted in significant increases in mean myofiber cross-sectional area in the EOM, the duration of which can be controlled by pellet manufacture. Thus IGF-1 treatment is optimized and side effects are minimized when the growth factor can be targeted to a specific muscle or group of muscles.

Our laboratory was the first to demonstrate the efficacy of single injections of IGF-1 and -2 in increasing muscle force generation in the adult extraocular muscles of mammals.^{7,8} This approach is supported by a study by other investigators that showed increased force generation after a retrobulbar injection of a cocktail of growth factors that included IGF-1 in developing chick extraocular muscles.⁹ However, it should be pointed out that this study examined force changes in developing muscle, while our study examined adult muscles.

The present study represents the first use of a sustained release device to maintain IGF-1 levels and to maintain increased muscle force during a 3-month period. It is clear that by 6 months, 3 months after the IGF-1 release ceases, force generation in the treated muscles returns to normal control levels. Single injections of IGF-1 result only in a

Table 2. Force measurements in mN/cm²

Stimulation frequency	Control	One month	Two months	Three months	Six months
Twitch	45.8 ± 5.5	79.0 ± 10.1	59.0 ± 14.5	82.3 ± 2.1	50.2 ± 8.9
10 Hz	47.2 ± 5.0	85.4 ± 11.4	61.1 ± 14.1	73.4 ± 1.8	52.9 ± 8.65
20 Hz	54.7 ± 5.44	97.6 ± 12.6	71.9 ± 17.2	84.5 ± 3.4	60.0 ± 9.3
40 Hz	87.13 ± 8.6	162.3 ± 22.0	114.9 ± 30.7	138.0 ± 3.4	90.7 ± 14.0
100 Hz	227.6 ± 19.7	461.3 ± 80.2	291.7 ± 60.2	348.7 ± 15.4	246.0 ± 32.3
150 Hz	271.4 ± 24.1	567.6 ± 78.2	339.2 ± 66.6	428.2 ± 22.1	297.6 ± 37.2
200 Hz	273.9 ± 25.4	32.9 ± 2.6	337.4 ± 62.9	439.6 ± 20.3	298.9 ± 38.5

Table 3. Twitch characteristics after 1 or 3 months of IGF exposure

Properties of single twitch	Injected 1 month	Control 1 month	Injected 3 months	Control 3 months
Total contraction time (ms)	193.3 ± 55.5	253.8 ± 103.5	258.2 ± 76.5*	258.2 ± 76.5*
Time to peak force (ms)	17.8 ± 6.6	27.0 ± 9.1	26.5 ± 1.8	30.8 ± 3.0
Half-relaxation time (ms)	13.0 ± 1.6	12.8 ± 1.5	15.0 ± 1.9*	12.7 ± 1.0
Max dF/dt (g/s)	626.0 ± 81.6*	533.9 ± 83.7	889.5 ± 95.6*	760.2 ± 70.8
Time to max dF/dt (ms)	6.3 ± 2.8*	20.8 ± 5.8	17.5 ± 1.8*	22.8 ± 3.0

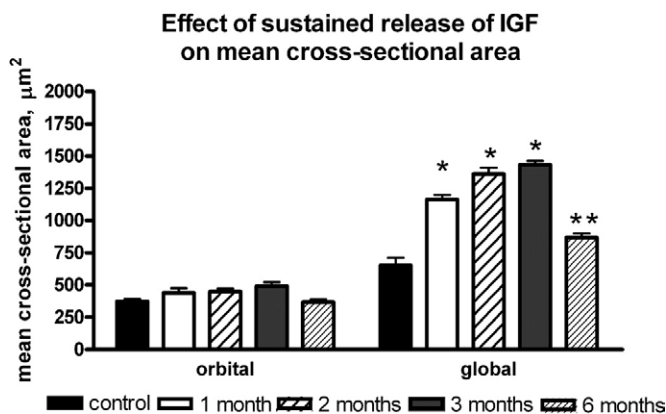


FIG 4. Mean cross-sectional area of the SR muscles 1, 2, 3, or 6 months after implantation of the IGF-1 or placebo pellets. *Significant difference from control animals.

brief increase in force generation. The importance of sustained increases in force generation is underlined by human studies which show that binocular vision and stereopsis does not develop properly in infants and children with strabismus if the eyes are not properly aligned and aligned for a sufficient duration to prevent loss of cortical binocularity.¹⁸⁻²¹ However, it is unclear how long binocular alignment is needed to maintain stability in eye position. It may be that the “failure rate” in botulinum toxin therapy is caused solely by those individuals for whom the effects wane most rapidly.⁵ However, the interaction of factors that contribute to this failure rate is complex and may relate to the type of strabismus, the particular patient response, and/or the timing of treatment relative to the appearance of the strabismus.^{20,22}

One of the unsolved problems in strabismus treatment of infants and children is that the duration of alteration of muscle strength needed to result in permanent correction is unknown, especially at a time when both motor and

sensory systems are developing; in other words, how long is “long enough”? To maximize the attainment of binocular vision and stereopsis, studies show that in the treatment of childhood strabismus, alignment should be accomplished within the first 2 years of life for maximum efficacy,^{23,24} and this has been confirmed in animal studies.²⁵ The rationale for the increased success of early treatment is based on the presence of a critical period for binocular sensory development in the developing visual cortex.^{26,27} Strabismus results in a loss of binocularity from the loss of neurons that are directly excitable by both eyes.^{28,29} During this critical period, cortical changes are extremely rapid,³⁰ making the time course for creating stable alterations more difficult to assess, particularly in human strabismic patients.

Some basis for estimating this time course can be gleaned from visual deprivation studies in animal models. One of the most powerful studies in defining parameters for duration of effect showed that central nervous system alterations from visual deprivation were not seen after 1 month of deprivation but required 3 months of visual deprivation.³¹ Two months of binocular visual experience did not reconstitute the normal visual system connectivity after these 3 months of visual deprivation. Using a different visual deprivation paradigm, only animals that suffered from visual deprivation for 40 and 55 days developed a persistent strabismus.³² This evidence suggests a hypothetical window for maintaining an alteration in extraocular muscle force of approximately 3 months. Further studies are needed to confirm or refute this hypothesis. Regardless, the sustained-release pellets are capable of maintaining IGF-1 release throughout this theoretical window.

In adult onset strabismus, the needed duration of treatment effect likely depends on the inciting pathology. However, duration should be sufficient to reestablish sustainable fusion, and that may require weeks to months of

treatment effect. Depending on the pathology, the combination of long-lasting agents that increase force generation with agents that weaken the antagonist may be needed.

As yet, there are no established pharmacologic options for strengthening underacting muscles in patients with strabismus. The data from this study and from our previous work certainly suggest that the use of growth factors is a feasible approach to accomplish this. Furthermore, sustained release of growth factors that increase muscle force generation in an underacting muscle could be used simultaneously with botulinum toxin or other muscle weakening agents placed into an overacting antagonist muscle. Altering the motive forces of agonist-antagonist pairs could allow titratable and sustained changes in the rotational position of the globe, which is the goal of strabismus surgery, and this may be accomplished without requiring an incisional procedure.

References

1. Livir-Rallatos G, Gunton KB, Calhoun JH. Surgical results in large-angle exotropia. *J AAPOS* 2002;6:77-80.
2. Trigler L, Siatkowski RM. Factors associated with horizontal reoperation in infantile esotropia. *J AAPOS* 2002;6:15-20.
3. Clark RA, Demer JL. Rectus extraocular muscle pulley displacement after surgical transposition and posterior fixation for treatment of paralytic strabismus. *Am J Ophthalmol* 2002;133:119-28.
4. Scott AB. Botulinum toxin injection into extraocular muscles as an alternative to strabismus surgery. *Ophthalmology* 1980;87:1044-9.
5. Tejedor J, Rodriguez JM. Long-term outcome and predictor variables in the treatment of acquired esotropia with botulinum toxin. *Invest Ophthalmol Vis Sci* 2001;42:2542-6.
6. Christiansen SP, Becker BA, Iaizzo PA, McLoon LK. Extraocular muscle force generation after ricin-mAb35 injection: implications for strabismus treatment. *J AAPOS* 2003;7:1-6.
7. McLoon LK, Christiansen SP. A novel approach to treatment of strabismus: increasing extraocular muscle strength in rabbits with insulin growth factor II. *Invest Ophthalmol Vis Sci* 2003;44:3866-72.
8. Anderson B, Christiansen SP, Grandt S, Grange RW, McLoon LK. Increased extraocular muscle strength with direct injection of insulin-like growth factor-I. *Invest Ophthalmol Vis Sci* 2006;47:2461-7.
9. Chen J, von Bartheld CS. Role of exogenous and endogenous trophic factors in the regulation of extraocular muscle strength during development. *Invest Ophthalmol Vis Sci* 2004;45:3538-45.
10. Barton ER, Morris L, Musaro A, Rosenthal N, Sweeney HL. Muscle-specific expression of insulin-like growth factor I counters muscle decline in mdx mice. *J Cell Biol* 2002;157:137-47.
11. Barton-Davis ER, Shoturma DI, Musaro A, Rosenthal N, Sweeney HL. Viral mediated expression of insulin-like growth factor I blocks the aging-related loss of skeletal muscle function. *Proc Natl Acad Sci USA* 1998;95:15603-7.
12. Lynch GS, Cuffe SA, Plant DR, Gregorevic P. IGF-I treatment improves the functional properties of fast- and slow-twitch skeletal muscles from dystrophic mice. *Neuromusc Disord* 2001;11:260-8.
13. Gregorevic P, Plant DR, Leeding KS, Bach LA, Lynch GS. Improved contractile function of the mdx dystrophic mouse diaphragm muscle after insulin-like growth factor-I administration. *Am J Pathol* 2002;161:2263-72.
14. Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, et al. Localized IGF-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat Genet* 2001;27:195-200.
15. Mulligan K, Schambelan M. Anabolic treatment with GH, IGF-1 or anabolic steroids in patients with HIV-associated wasting. *Int J Cardiol* 2002;85:151-9.
16. Goldspink G. Impairment of IGF-I gene splicing and MGF expression associated with muscle wasting. *Int J Biochem Cell Biol* 2006;38:481-9.
17. Thoenen H, Sendtner M. Neurotrophins: from enthusiastic expectations through sobering experiences to rational therapeutic approaches. *Nature Neurosci* 2002;5:1046-50.
18. Tychsen L, Wong AMF, Burkhalter A. Paucity of horizontal connections for binocular vision in VI of naturally strabismic macaques: cytochrome oxidase compartment specificity. *J Comp Neurol* 2004;474:261-75.
19. Birch EE, Petrig B. FPL and VEP measures of fusion, stereopsis and stereoacuity in normal infants. *Vision Res* 1996;36:1321-7.
20. Birch EE, Stager DR, Berry P, Leffler J. Stereopsis and long-term stability of alignment in esotropia. *J AAPOS* 2004;8:146-50.
21. Wong AMF, Burkhalter A, Tychsen L. Suppression of metabolic activity caused by infantile strabismus and strabismic amblyopia in striate visual cortex of Macaque monkeys. *J AAPOS* 2005;9:37-47.
22. Spencer RF, McNeer KW. Botulinum toxin paralysis of adult monkey extraocular muscle. Structural alterations in orbital, singly innervated muscle fibers. *Arch Ophthalmol* 1987;105:1703-11.
23. Birch EE, Fawcett S, Stager DR. Why does early surgical alignment improve stereoacuity outcomes in infantile esotropia? *J AAPOS* 2000;4:10-14.
24. Ing MR. Early surgical alignment for congenital esotropia. *Ophthalmology* 1983;90:132-5.
25. Wong AMF, Foeller P, Bradley D, Burkhalter A, Tychsen L. Early versus delayed repair of infantile strabismus in macaque monkeys. I. Ocular motor effects. *J AAPOS* 2003;7:200-9.
26. Hubel DH, Wiesel TN. Single-cell responses in striate cortex of kittens deprived of vision in one eye. *J Neurophysiol* 1963;26:1003-17.
27. Hubel DH, Wiesel TN. Binocular interaction in striate cortex of kittens reared with artificial squint. *J Neurophysiol* 1965;28:1041-59.
28. Blakemore C. The conditions required for the maintenance of binocularly in the kitten's visual cortex. *J Physiol Lond* 1976;261:423-44.
29. Sengpiel F, Blakemore C, Kind PC, Harrad R. Interocular suppression in the visual cortex of strabismic cats. *J Neurosci* 1994;14:6855-71.
30. Trachtenberg JT, Stryker MP. Rapid anatomical plasticity of horizontal connections in the developing visual cortex. *J Neurosci* 2001;21:3476-82.
31. Innocenti GM, Frost DO, Illes J. Maturation of visual callosal connections in visually deprived kittens: a challenging critical period. *J Neurosci* 1985;5:255-67.
32. Tusa RJ, Mustari MJ, Burrows AF, Fuchs AF. Gaze-stabilizing deficits and latent nystagmus in monkeys with brief, early-onset visual deprivation: eye movement recordings. *J Neurophysiol* 2001;86:651-61.