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Distribution and appearance of myosin, dystrophin, and collagen IV in strabismus-affected extraocular muscle tissue compared with control tissue

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Abstract

Objective: Extraocular muscles have complex development processes. The present study aimed to analyze the presence of myosin, dystrophin, and collagen IV in the strabismus-affected extraocular muscle.

Methods: This research was an observational case–control study. Myosin, dystrophin, and collagen IV were detected by histological and immunohistochemical analyses of extraocular muscle samples from concomitant strabismus patients and controls. A semi-quantitative grading method and statistical analysis were used.

Results: In the strabismus-affected extraocular muscle, morphological analysis demonstrated different-sized muscle fibers. Immature muscle fibers and an increased amount of connective tissue were also noted. Strong positive correlations were identified between myosin and collagen IV and between dystrophin and collagen IV.

Conclusions: The presence of newly formed muscle fibers, increased connective tissue, and variable diameters of skeletal striated muscle fibers indicate the decreased quality of extraocular muscles in strabismus cases. Reduced levels of myosin and dystrophin and a near absence of collagen IV in strabismus-affected skeletal striated muscle fibers characterized the muscular dystrophy of strabismus. Adjuvant therapy aimed at normalizing the metabolism of these muscles may be appropriate alongside concomitant strabismus treatment.

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Introduction

Strabismus, also known as squint, is a visual condition characterized by the misalignment of the visual axes.[1] This condition commonly manifests in childhood but can occur at any stage in life, and is one of the most prevalent eye disorders in children and teenagers. Strabismus can lead to physical discomfort and malfunction in binocular vision, potentially impacting the overall quality of life.[2,3] Despite numerous theories concerning its origins, the precise etiology of strabismus remains elusive.

Strabismus can be classified as concomitant or noncomitant. In the case of concomitant strabismus, the size of the deviation between eyes does not vary with the direction of gaze. Conversely, noncomitant strabismus is usually the result of a limitation in eye movement that is associated with a paralytic or mechanical etiology.[4] Surgical treatment is believed to improve the quality of life of patients with some types of strabismus,[5,6] but does not achieve a long-lasting effect in all cases, similar to other therapeutic measures.[7,8] The development of new methods to treat strabismus thus remains an urgent need.[9] To clarify the extent to which it may be worthwhile to develop additional methods of strabismus treatment aimed at improving the metabolism of the external muscles of the eyeball, we conducted morphological studies of the state of these muscles in individuals suffering from concomitant (congenital) strabismus.

The six extraocular muscles consist of skeletal striated muscle fibers that are evenly spaced, uniform in size, and separated by connective tissue; they enable the coordinated movement of the eyes. Pathological changes in extraocular muscles, including disarrangement, degeneration, and atrophy of skeletal striated muscle fibers, have been reported. Moreover, immature skeletal striated muscle fibers, indicating regeneration, have been observed along with inflammatory cell infiltration, loss of striation, and excessive extracellular matrix among these muscle fibers.[10,11] Distinct thicknesses, internalized nuclei, and abnormal alignments of skeletal striated muscle fibers have been identified in extraocular muscles affected by strabismus. These pathological alterations are exacerbated by age and chronic strabismus.[12] Increased myofiber size and a decreased number of satellite cells have also been noted in patients with intermittent exotropia.[13]

Protein filaments such as myosin and dystrophin can serve as indicators of the quality of skeletal striated muscle fibers. Myosin is a cytoskeletal motor protein that has 12 classes; it plays a vital role in muscle tissue contraction and movement alongside actin filaments.[14] Myosin participates in a variety of cellular activities, including cell adhesion and signaling, intracellular transport, formation of membrane protrusions, and cell migration; a wide range of diseases are therefore associated with myosin defects.[15] Dystrophin is associated with the sarcolemma in skeletal striated muscle tissue, and is part of a protein complex that spans the membrane and connects the cytoskeleton to the basement membrane. By interacting with various integral membrane proteins, dystrophin

stabilizes the sarcolemma and participates in the transmembrane signaling complex.[15,16] Given these roles, it is conceivable that changes in myosin or dystrophin may be involved in the etiology of strabismus.

Collagen IV is an essential component of the basal membrane surrounding and supporting skeletal striated muscle fibers, and is made up of six proteins encoded by six distinct genes. Collagen IV maintains basal membrane integrity, cellular organization, and filtration functions.[17,18] A deficiency in collagen IV in mouse embryos leads to altered basement membrane structures, highlighting its crucial role in maintaining the stability of the basement membrane.[19] Alterations in collagen IV synthesis and degradation in skeletal striated muscle tissue have been observed during immobilization.[20] Collagen IV may therefore serve as a tool for evaluating potential changes in the basement membrane.

Given the limited number of existing studies on this subject, the objective of the current study was to investigate the distribution and appearance of myosin, dystrophin, and collagen IV in extraocular muscle tissue from patients with strabismus compared with control tissue. The novelty of the research presented herein lies primarily in its pioneering approach to understand the underpinnings of strabismus, a common eye condition. Notably, the research brings into focus the role of key proteins—myosin, dystrophin, and collagen IV—that contribute to the structure and functioning of muscle fibers. These proteins have been studied previously in the context of their role in muscle tissue, but their potential involvement in strabismus is a novel concept.

In the present study, we also introduce the idea that strabismus may result from a primary extraocular muscle abnormality, thus challenging the traditional belief that strabismus is largely a secondary manifestation of other ocular conditions. This novel perspective may redefine our understanding of and approaches toward the diagnosis and treatment of strabismus.

In summary, the novelty of the present study lies in its unique approach, the important findings on the role of muscle fiber proteins in strabismus, and its forward-thinking ideas for future research in the field. The findings presented will contribute to the evolution of our understanding and management of strabismus.

Methods

We conducted an observational case–control study in accordance with the Declaration of Helsinki. The study was approved by the Ethical Committee of Rīga Stradiņš University on 19 October 2021 (no. 22-2/465/2021). Written informed consent was obtained from all subjects involved in the study. All patient information was encrypted and cannot be identified without the key. The reporting of this study conforms to STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.[21]

We collected extraocular muscle samples from concomitant (congenital) strabismus patients. Exclusion criteria encompassed strabismus resulting from other causes and existing conditions affecting the eye or orbital structures. The control group comprised extraocular muscle samples that were acquired post-mortem from autopsy cases. These cases were all individuals who had passed away as the result of accidents and who had no recorded systemic or eye diseases in their medical history.

All tissue used in the present study was obtained at around the same time, and all samples underwent identical staining, fixation, and embedding procedures to maintain consistency. The assembled material for this research was collected under the stewardship of the Institute of Anatomy and Anthropology at Rīga Stradiņš University.

All biopsy samples were fixed with 2% formaldehyde and 0.2% picric acid in 0.1 M phosphate buffer (pH 7.2) for 24 hours. After fixation, the samples were rinsed in Tyrode's buffer for 12 hours before being embedded in paraffin. Subsequently, 4-μm sections were cut and stained with hematoxylin and eosin for routine morphological structure evaluation.

Immunohistochemical analysis

The biotin–streptavidin biochemical method was used for the detection of myosin (ab7784, diluted 1:150; Abcam, Cambridge, UK), dystrophin (ab15277, diluted 1:100; Abcam), and collagen IV (520369 A, diluted 1:30; Invitrogen Corp., Carlsbad, CA, USA). Immunoreactive structures were evaluated in skeletal striated muscle tissue in 10 random visual fields at 400× (ocular 10×, objective 40×). Each evaluation was conducted by two researchers to avoid subjectivity. Median values were used for further data analysis. The stained slides were examined under a light microscope (Leica DC300F; Leica Biosystems, Richmond, VA, USA). For image processing, visual illustration, and analysis, Image-Pro Plus software (Media Cybernetics, Inc., Rockville, MD, USA) was used.

Quantification of immunoreactive cells

A semiquantitative grading method was used to evaluate the appearance and distribution of the immunoreactive structures of myosin, dystrophin, and collagen IV. The semiquantitative grading method used the following labels: 0, negative staining (0%); 0/+, occasional positive structures (12.5%); +, few positive structures (25%); +/++, few to moderate number of positive structures (37.5%); ++, moderate number of positive structures (50%); ++/+++, moderate to numerous positive structures (62.5%); +++, numerous positive structures (75%); +++/++++, numerous to abundant positive structures (87.5%); and ++++, an abundance of positive structures (up to 100%) in the visual field.[22]

Statistical analysis

IBM SPSS Statistics for Windows, version 27.0 (IBM Corp., Armonk, NY, USA) was used to perform all statistical analyses of the data. The results from the semiquantitative grading method were transformed into SPSS as follows: negative staining, 0; occasional stained structures, 0.5; few stained structures, 1.0; few to moderate number of stained structures, 1.5; moderate number of stained structures, 2.0; moderate to numerous stained structures, 2.5; numerous stained structures, 3.0; numerous to abundant stained structures, 3.5; and abundant stained structures, 4.0.

Nonparametric statistical methods were used to statistically analyze the data. The Mann–Whitney *U* test was applied to determine significant differences between the patient and control groups.[23] To detect correlations between different factors in the patient group, Spearman's rank correlation coefficient *rs* (Spearman's rho) was calculated.[24] The correlation coefficient (*rs*) was interpreted as follows: 0.80 to 1.00, very high; 0.60 to 0.79, high; 0.40 to 0.59, moderate; 0.20 to 0.39, low; and <0.2, very low correlation. For all tests, $p \leq 0.05$ was considered significant.

Results

The patient group comprised extraocular muscle samples from 10 concomitant strabismus patients (six male and four female) ranging in age from 7 to 60 years. Of these samples, seven were procured from the rectus medialis muscle and three from the rectus lateralis muscle. The control group comprised extraocular muscle samples from five autopsy cases (two men and

three women) aged between 30 and 57 years. Two samples were from the rectus medialis muscle and three were from the rectus lateralis muscle.

The morphological analysis of the strabismus-affected extraocular muscles demonstrated different-sized skeletal striated muscle fibers with various diameters. Newly formed (i.e., immature) skeletal striated muscle fibers and an increased amount of connective tissue were also noted (Figure 1a, b).

Structures immunoreactive for myosin, dystrophin, and collagen IV were observed in all tissue samples. On closer examination, differences between the patient and control groups were revealed. The median values of myosin, dystrophin, and collagen IV all showed a higher number of positive structures in controls than in patients. Furthermore, relatively small fluctuations were observed between myosin-, dystrophin-, and collagen IV-immunoreactive structures in both the patient and control groups (Table 1).

There was a moderate number of myosin-positive skeletal striated muscle fibers in the patient group. However, some samples presented a few to a moderate number of myosin-immunoreactive structures. In the control group, there were more myosin-positive muscle fibers than in the patient group; these had a very stable appearance, with moderate to numerous positive muscle fibers (Table 1, Figure 2a, b).

There was a few to a moderate number of dystrophin-immunoreactive structures in the muscle fibers of the patient group. However, four patient samples had a moderate number of dystrophin-positive skeletal striated muscle fibers. In the control group, the median value for dystrophin was similar to the median value for myosin (Table 1, Figure 2c, d).

Collagen IV-immunoreactive structures were almost absent in strabismus-affected extraocular muscles; they were occasionally noted in the sarcolemma of skeletal striated muscle fibers. In one patient sample only, a few collagen IV-positive structures were detected. By contrast, the control group had a stable expression of a moderate number of myosin-positive structures (Table 1, Figure 2e, f).

The Mann–Whitney *U* test revealed significant differences between the patient and control groups in the following proteins:

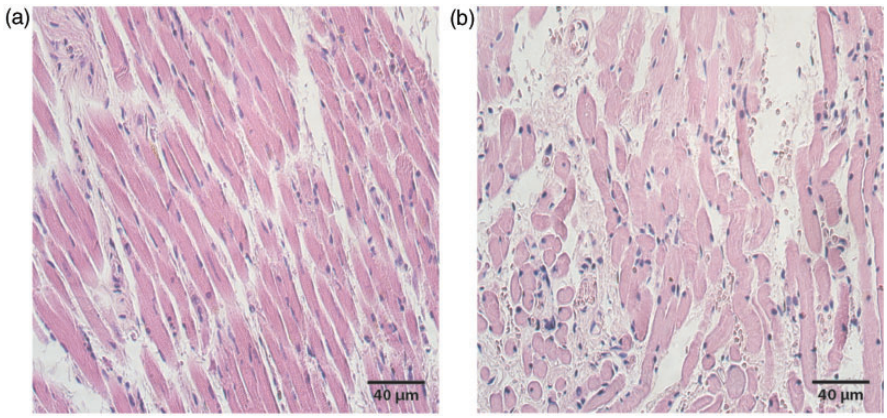

Figure 1. Hematoxylin and eosin staining of control and strabismus-affected muscle tissue (250× magnification). (a) Unaltered skeletal striated muscle fibers were observed in the control group and (b) There were skeletal striated muscle fibers of variable diameter and increased amounts of connective tissue in strabismus-affected extraocular muscle tissue.

Table 1. Median values of the semiquantitative evaluation of myosin-, dystrophin-, and collagen IV-positive structures in the patient and control groups.

Participant Code	Myosin	Dystrophin	Collagen IV
P1	+/++	+/++	0/+
P2	++	+/++	0/+
P3	++	++	0/+
P4	++	+/++	0/+
P5	++	++	0/+
P6	++	+/++	0/+
P7	++	++	+
P8	++	+/++	0/+
P9	+/++	+/++	0/+
P10	+/++	++	0/+
Median (P)	++	+/++	0/+
C1	++/+++	++/+++	+/++
C2	++/+++	++	++
C3	++	++/+++	++
C4	++	++/+++	++
C5	++/+++	++	++
Median (C)	++/+++	++/+++	++

C1–C5, median value in each control sample; Median (C), median value in the control group sample; Median (P), median value in the patient group sample; P1–P10, median value in each patient sample; 0/+, occasional positive structures; +, a few positive structures; +/++, a few to a moderate number of positive structures; ++, a moderate number of positive structures; ++/+++, a moderate number to numerous positive structures.

myosin (Mann–Whitney $U = 7.0$; Z-score $= -2.510$; $p = 0.028$), dystrophin (Mann–Whitney $U = 4.0$; Z-score $= -2.761$; $p = 0.008$), and collagen IV (Mann–Whitney $U = 0$; Z-score $= -3.494$; $p = 0.001$). Moreover, in the patient group, two high positive correlations were detected: between myosin and collagen IV ($rs = 0.640$; $p = 0.010$) and between dystrophin and collagen IV ($rs = 0.748$; $p = 0.001$).

Discussion

In the present study, we observed alterations in the extraocular muscles as well as changes in the expression of myosin, dystrophin, and collagen IV that were consistent across all patients. To assess the potential roles of myosin, dystrophin, and collagen IV in the development of strabismus, comparisons were then drawn between the patient and control groups in terms of immunoreactive structures. All investigated markers were significantly lower in the patient group than in the control group ($p < 0.05$), indicating the presence of a greater number of positive structures in the control group.

We postulate that the presence of abundant connective tissue, differing diameters of skeletal striated muscle fibers, and newly formed muscle fibers are nonspecific changes in extraocular muscle tissues affected by strabismus; they potentially result from typical muscle alterations caused by irritation, a common muscular response to various stimuli.[25] In the patient group, we observed strong positive correlations between myosin and collagen IV, and between dystrophin and collagen IV. These findings, along with the reduced quality and number of structures positive for myosin, dystrophin, and collagen IV within skeletal striated muscle fibers in strabismus-affected extraocular muscles, hint at serious tissue damage associated with the disease. The observed changes were not dependent on age or the specific extraocular muscle affected by strabismus.

Contraction speed and fatigue resistance are crucial to the functional characteristics of muscle tissue, and other studies have linked these properties to muscle fiber structure.[26,27] Myosin influences muscle fiber contraction speed and is crucial for muscle tissue contraction functions, its role within the sarcomere, and its interaction with other filaments.[28,29] The diverse cellular activities of myosin thus underscore its importance beyond contraction, to the physiological functionality and sustainability of skeletal striated muscle tissue.[14,30]

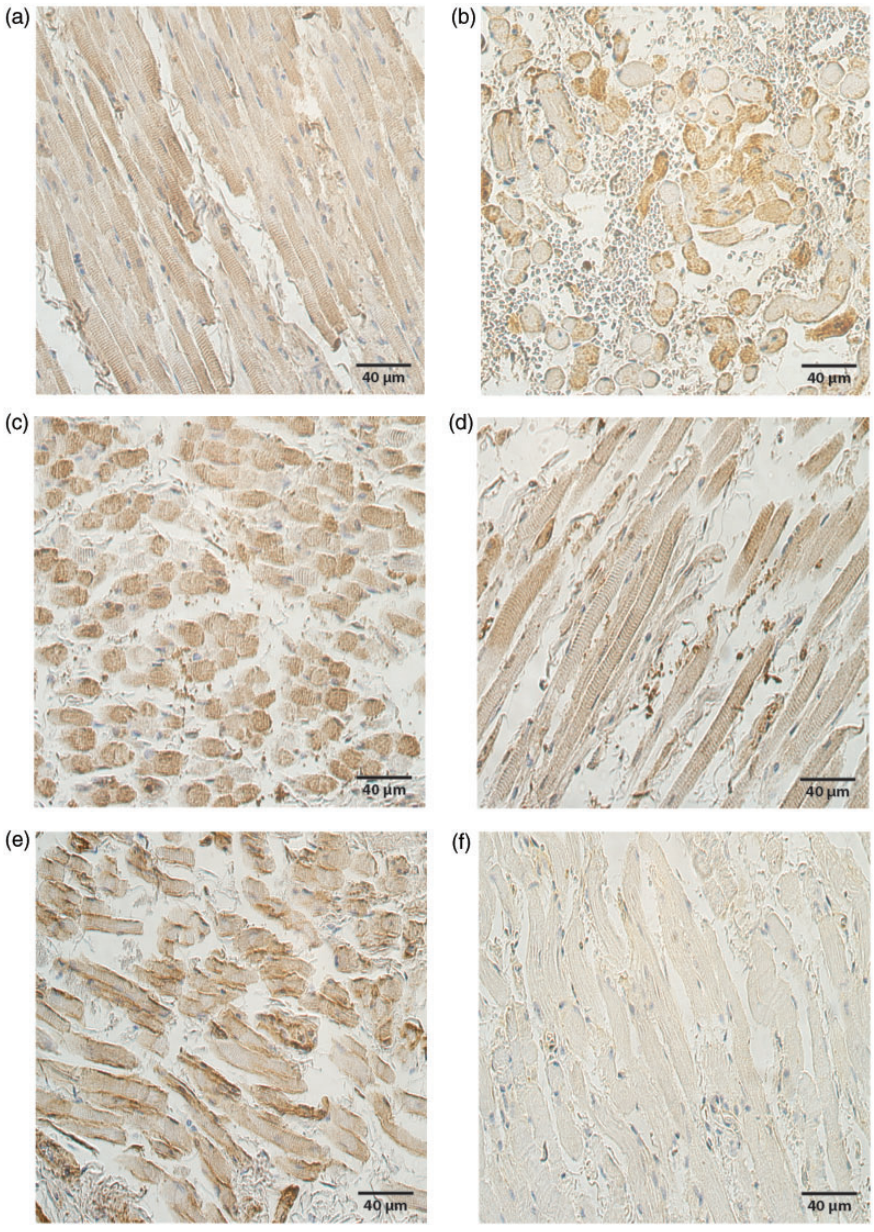

Figure 2. Immunoreactive structures in control and strabismus-affected muscle tissue (250× magnification). (a) There was a moderate number (++) of myosin-positive skeletal striated muscle fibers in the control group. (b) There was a moderate number (++) of myosin-positive skeletal striated muscle fibers in strabismus-affected extraocular muscle. (c) There was a moderate to numerous number (++/+++) of dystrophin-positive skeletal striated muscle fibers in the control group. (d) There were a few to a moderate number (+/++) of dystrophin-positive skeletal striated muscle fibers in strabismus-affected extraocular muscle. (e) There was a moderate number (++) of collagen IV-positive sarcolemma in skeletal striated muscle fibers of the control group and (f) There were only occasional (0/+) collagen IV-positive sarcolemma (arrow) in skeletal striated muscle fibers of strabismus-affected extraocular muscle tissue.

Dystrophin is another critical component of muscle fiber functionality; it is linked to the subsarcolemmal cytoskeleton and various extracellular matrix proteins. Dystrophin is a major component of the dystrophin protein complex, interacting with various signaling proteins. This complex is vital for stabilizing the plasma membrane in skeletal striated muscle tissue.[31] Although the functions of dystrophin are not yet fully defined, studies have suggested that its absence can lead to membrane damage and the disruption of skeletal striated muscle signaling cascades.[15,16,32] These findings are supported by the strong correlations that we noted in the patient group between myosin and collagen IV, and between dystrophin and collagen IV.

An important finding in the present study was the significant decrease in dystrophin expression in strabismus-affected extraocular muscle tissue compared with that of healthy controls. This reduction was not dependent on the age of the patients nor the side of the extraocular muscle affected by strabismus, thus highlighting the role of dystrophin deficiency as a potential causative or contributory factor in the development of strabismus. Also intriguing was the observation of a strong correlation between the expression of dystrophin and collagen IV, which is another vital protein for muscle tissue maintenance. This finding indicates a potential interconnected dysfunction or dysregulation in the expression of these proteins in strabismus-affected tissues. However, our findings also suggest that, although dystrophin has an essential role in maintaining muscle integrity, its exact function—and the ramifications of its decreased expression in strabismus—are not yet fully understood. Further research is needed to better understand the complexities of the function of dystrophin and its role in strabismus pathogenesis. It should also be noted that our results were based on the examination of the C-terminus domain of dystrophin only; the conclusions may therefore not present a complete picture of the role of dystrophin in the pathogenesis of strabismus. A more comprehensive understanding may be achieved by also examining the N-terminus and rod-domain of dystrophin. Nonetheless, our study suggests a potential role of dystrophin expression changes in strabismus, although the complexity of dystrophin's function and its interaction with other proteins necessitates more detailed studies to fully elucidate its involvement in this common eye disorder.

Although the theory that strabismus results from primary extraocular muscle abnormalities is not yet universally accepted, our data indicate a significant decrease in myosin and dystrophin within strabismus-affected extraocular muscle tissue. We also observed altered muscle tissue morphology in the patient group during routine histological analyses. Combined with the decreased myosin- and dystrophin-immunoreactive structures within patient tissue, these findings suggest that muscular dystrophy is characteristic of persistent strabismus.

Collagen IV is a major basement membrane component that is essential for maintaining the plasma membrane integrity of skeletal striated muscle tissue. Collagen IV enhances the stability of the basement membrane and maintains its structural integrity.[33,34] Notably, among all of the markers that we examined, collagen IV had the fewest immunoreactive structures in tissue from patients. This finding suggests that a decrease in collagen IV-positive sarcolemma in the patient group indicates damage to skeletal striated muscle fibers. This conclusion is also supported by the strong correlations in the patient group between myosin and collagen IV, and between dystrophin and collagen IV.

The present study is not without limitations. The investigation of skeletal striated muscle fiber quality-related proteins and

the main components of sarcolemma in strabismus-affected extraocular muscle tissue remains a relatively unexplored area. Future studies should use additional methods, such as *in situ* hybridization and enzyme-linked immunosorbent assays, to assess strabismus pathogenesis from multiple perspectives, including gene/gene–protein involvement and the quantification of tissue factor concentration changes. An expansion of the research cohort, to include both children and adults affected by strabismus, is also planned. Future research should also explore tissue remodeling and angiogenetic factors in strabismus-affected skeletal striated muscle fibers using immunocytochemistry.

In conclusion, the presence of newly formed skeletal striated muscle fibers, an increased amount of connective tissue, and variable diameters of skeletal striated muscle fibers in the present study indicate the decreased quality of strabismus-affected extraocular muscles. The diminished myosin and dystrophin in strabismus-affected skeletal striated muscle fibers, and the near absence of collagen IV, characterize the muscular dystrophy that occurs in strabismus. Our findings also demonstrated strong correlations between skeletal striated muscle fiber quality-related proteins and the main component of sarcolemma (i.e., collagen IV). The present detection of morphological changes in the muscles of the eyeball—the function of which is weakened in patients with strabismus—indicates that adjuvant therapy aimed at normalizing the metabolism of these muscles may be appropriate in combination with conjugate strabismus treatment in the future.

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Author contributions

Conceptualization, M.P.; methodology, M.P.; software, all authors; validation, all authors; formal analysis, A.J. and M.P.; investigation, all authors; resources, T.B., P.F., and M.P.; data curation, all authors; writing, original draft preparation, A.J.; writing – review and editing, all authors; visualization, A.J. and M.P.; supervision, M.P. All authors have read and agreed to the published version of the manuscript.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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References

1. Gunton KB, Wasserman BN and DeBenedictis C. Strabismus. *Prim Care* 2015; 42: 393–407.
2. Hatt SR, Leske DA, Kirgis PA, et al. The effects of strabismus on quality of life in adults. *Am J Ophthalmol* 2007; 144: 643–647.
3. Kumaran SE, Khadka J, Baker R, et al. Patient-reported outcome measures in amblyopia and strabismus: a systematic review. *Clin Exp Optom* 2018; 101: 460–484.
4. Sunyer-Grau B, Quevedo L, Rodriguez-Vallejo M, et al. Comitant strabismus etiology: extraocular muscle integrity and central nervous system involvement—a narrative review. *Graefes Arch Clin Exp Ophthalmol* 2023; 261: 1781–1792.
5. Huang Y, Li K, Yan Y, et al. Strabismus surgery for improving the quality of life of strabismus patients with thyroid-associated ophthalmopathy. *Medicine (Baltimore)* 2023; 102: e34778.
6. Jellema HM, Merckel-Timmer E, Kloos R, et al. Life quality improves after strabismus

surgery in patients with Graves' orbitopathy. *Eur J Endocrinol* 2014; 170: 785–789.
7. Pineles SL, Repka MX, Yu F, et al. Risk of musculoskeletal injuries, fractures, and falls in Medicare beneficiaries with disorders of binocular vision. *JAMA Ophthalmol* 2015; 133: 60–65.
8. Lueder GT, Galli M, Tychsen L, et al. Long-term results of botulinum toxin-augmented medial rectus recessions for large-angle infantile esotropia. *Am J Ophthalmol* 2012; 153: 560–563.
9. Ozkan SB, Kir E, Dayanir V, et al. Botulinum toxin A in the treatment of adherence syndrome. *Ophthalmic Surg Lasers Imaging* 2003; 34: 391–395.
10. Hamdi MM, El-Hawary GR, El-Hefnawy NG, et al. Histopathological and electron microscopic study for different grades of inferior oblique muscle overaction. *Clin Ophthalmol* 2013; 7: 917–921.
11. Al-Falki Y, Al-Shraim M, Alsabaani NA, et al. Ultrastructural changes of extraocular muscles in strabismus patients. *Ultrastruct Pathol* 2019; 43: 145–153.
12. Yao J, Wang X, Ren H, et al. Ultrastructure of medial rectus muscles in patients with intermittent exotropia. *Eye (Lond)* 2016; 30: 146–151.
13. Kim CZ and Lee SJ. Increased myofiber size and reduced satellite cell numbers in medial rectus muscle of patients with intermittent wxotropia. *Strabismus* 2020; 28: 201–207.
14. Masters TA, Kendrick-Jones J and Buss F. (2016). Myosins: domain organisation, motor properties, physiological roles and cellular functions. In: BM Jockusch (Ed.) *The Actin Cytoskeleton, Handbook of Experimental Pharmacology*. Springer International Publishing: Cham, 2016, pp. 77–122.
15. Muntoni F, Torelli S and Ferlini A. Dystrophin and mutations: one gene, several proteins, multiple phenotypes. *Lancet Neurol* 2003; 2: 731–740.
16. Blake DJ, Weir A, Newey SE, et al. Function and genetics of dystrophin and dystrophin-related proteins in muscle. *Physiol Rev* 2002; 82: 291–329.
17. Khoshnoodi J, Pedchenko V and Hudson BG. Mammalian collagen IV. *Microsc Res Tech* 2008; 71: 357–370.
18. Bai X, Dilworth DJ, Weng YC, et al. Developmental distribution of collagen IV isoforms and relevance to ocular diseases. *Matrix Biol* 2009; 28: 194–201.
19. Pöschl E, Schlötzer-Schrehardt U, Brachvogel B, et al. Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. *Development* 2004; 131: 1619–1628.
20. Ahtikoski AM, Koskinen SOA, Virtanen P, et al. Synthesis and degradation of type IV collagen in rat skeletal muscle during immobilization in shortened and lengthened positions: immobilization and type IV collagen. *Acta Physiol Scand* 2003; 177: 473–481.
21. Von Elm E, Altman DG, Egger M, STROBE Initiative, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann Intern Med* 2007; 147: 573–577.
22. Pilmane M, Luts A and Sundler F. Changes in neuroendocrine elements in bronchial mucosa in chronic lung disease in adults. *Thorax* 1995; 50: 551–554.
23. Mann HB and Whitney DR. On a test of whether one of two random variables is stochastically larger than the other. *Ann Math Statist* 1947; 18: 50–60.
24. Mukaka MM. Statistics corner: a guide to appropriate use of correlation coefficient in medical research. *Malawi Med J* 2012; 24: 69–71.
25. Mukund K and Subramaniam S. Skeletal muscle: a review of molecular structure and function, in health and disease. *Wiley Interdiscip Rev Syst Biol Med* 2020; 12: e1462.
26. Porter MM, Vandervoort AA and Lexell J. Aging of human muscle: structure, function and adaptability. *Scand J Med Sci Sports* 2007; 5: 129–142.
27. Sweeney HL and Hammers DW. Muscle contraction. *Cold Spring Harb Perspect Biol* 2018; 10: a023200.
28. Reconditi M, Linari M, Lucii L, et al. Structure-function relation of the myosin motor in striated muscle. *Ann N Y Acad Sci* 2005; 1047: 232–247.

29. Lennerstrand G. Strabismus and eye muscle function. *Acta Ophthalmol Scand* 2007; 85: 711–723.
30. Coluccio LM. Myosins and disease. In: LM Coluccio (Ed.) *Advances in Experimental Medicine and Biology*. Springer International Publishing: Cham, 2020, pp. 245–316.
31. Gao QQ and McNally EM. The dystrophin complex: structure, function, and implications for therapy. In: R Terjung (Ed.) *Comprehensive Physiology*. Wiley., 2015, pp. 1223–1239.
32. Allen DG, Whitehead NP and Froehner SC. Absence of dystrophin disrupts skeletal muscle signaling: roles of Ca^{2+}, reactive oxygen species, and nitric oxide in the development of muscular dystrophy. *Physiol Rev* 2016; 96: 253–305.
33. LeBleu VS, MacDonald B and Kalluri R. Structure and function of basement membranes. *Exp Biol Med (Maywood)* 2007; 232: 1121–1129.
34. Pozzi A, Yurchenco PD and Iozzo RV. The nature and biology of basement membranes. *Matrix Biol* 2017; 57-58: 1–11.