

Histopathological Characteristics of the Orbicularis Oculi Muscle After Lower Blepharoplasty With or Without Myotomy

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Abstract Lower blepharoplasty is a challenging aesthetic procedure. Despite advances in clinical and neuroanatomical studies related to orbicularis oculi muscle innervation, no study has examined its histopathological aspects in different lower blepharoplasty procedures. This study aimed to assess changes in the pretarsal muscle complex in patients treated with transcutaneous lower blepharoplasty with orbicularis myotomy versus those treated with transconjunctival blepharoplasty without myotomy.

Methods A total of 268 patients underwent blepharoplasty performed by a single surgeon: transcutaneous lower blepharoplasty in 112 (41.7%) and transconjunctival lower blepharoplasty with retroseptal access in 156 (58.2%). Subsequent minor blepharoplasty procedures were performed in 32 patients with lower pretarsal orbicularis oculi muscle biopsy. Connective tissue, fibrillar elastic system, nerves, blood vessels, fiber diameter, and sarcomeres were analyzed.

Results Without myotomy: collagen, $9.46 \pm 0.41\%$; elastin, $7.03 \pm 0.62\%$; blood vessels, $4.25 \pm 0.06\%$; nerves, $5.2 \pm 0.55\%$; fiber diameter, $62 \pm 8.0 \mu\text{m}$; and sarcomere, $1.9 \pm 0.11 \mu\text{m}$. With myotomy: collagen, $19.02 \pm 1.66\%$; elastin, $7.88 \pm 1.10\%$; blood vessels, $5.13 \pm 0.70\%$; nerves, $2.9 \pm 0.48\%$; fiber diameter, $49 \pm 6.5 \mu\text{m}$; and sarcomere, $1.8 \pm 0.17 \mu\text{m}$.

Discussion Excess endomysial connective tissue, particularly between muscle fibers, should be considered pathological. The pathological changes in the pretarsal muscle complex after blepharoplasty with myotomy may have resulted from innervation interruptions as well as vascularization blockages.

Conclusions Surgeries with myotomy caused significant changes in collagen, nerves, and muscle fiber diameter. These observations strongly suggest the superiority of a technique that preserves orbicularis oculi muscle integrity. **Level of Evidence III** This journal requires that authors assign a level of evidence to each article. For a full description of these Evidence-Based Medicine ratings, please refer to the Table of Contents or the online Instructions to Authors www.springer.com/00266.

Keywords Pretarsal orbicular complex · Histopathology · Myotomy

Introduction

Blepharoplasty is one of the most common cosmetic surgeries. According to the American Society for Aesthetic Plastic Surgery [1], it was the third most frequent procedure performed in 2017 in the USA. Despite its frequency, lower blepharoplasty is recognized as one of the most challenging aesthetic procedures. Numerous surgical techniques have been described, and there is no consensus in the literature of the ideal procedure to correct the structural changes associated with aging [2–10].

Historically two types of lower palpebral aesthetic procedures have been frequently employed: the anterior transcutaneous approach and the posterior transconjunctival approach. In the traditional transcutaneous technique,

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the skin, muscle, and adipose tissue (eyelid pouches) are treated through an infraciliary incision. The dissection progresses through the orbicular musculature, exposing the orbital septum that is open to allow the exposure, removal, or transposition of the orbital fat. The anterior access method provides excellent exposure of the palpebral fat bags and leaves a well-positioned and barely visible final scar. Postoperative complications such as eyelid retraction and ectropion are relatively high at 15–20% [11–15]. These complications are related to the possibility of denervation of the orbicularis muscle and middle lamella retractions.

The acceptance of lower transconjunctival blepharoplasty has grown in recent years [8, 10, 16]. The main advantage of conjunctival access is preservation of the orbital septum and the orbicular muscle, which reduces the risk of changing the lower eyelid position [8, 16].

Recent neuroanatomical studies of the periorbital region clarified important aspects of the motor innervation of the lower eyelid orbicular muscle that modifies old concepts. Ramirez and Santamarina [17] and Hwang et al. [18] revealed terminal zygomatic branches penetrating the lower eyelid orbicular muscle in perpendicular position to their fibers. Lowe et al. [19] showed in cadavers the presence of multiple motor branches innervating the lower eyelid without identifying a single dominant branch. Bird and Burt [20] stated that the section of the terminal lateral motor nerve branches in transmuscular lower blepharoplasties contributed to the loss of pretarsal orbicularis tone, requiring complementary canthoplasty. Nemoto [21] performed neuroanatomical studies describing ascending branches of the buccal nerve innervating the orbicular palpebral muscle in its medial palpebral ligament as well as part of the corrugator and procerus muscles. These studies were confirmed by Mc Cord et al. [22], and Ouattara et al. [23] suggested that the section of the lower orbicularis muscle in blepharoplasties would not be responsible for “denervation” alterations since the motor innervation of the orbicularis has two main plexuses formed by the zygomatic and buccal branches. Di Francesco et al. [24] evaluated patients before and after transcutaneous blepharoplasty by performing electromyographic examinations and blink tests and recording videos. The results of this study showed that changes in position and inferior eyelid dysfunction after blepharoplasty cannot be explained by denervation of the zygomatic branch of the facial nerve and that any acute or residual denervation observed after subciliary and orbicular incisions is not clinically significant.

The most important recommendation for this operation is a perfect maneuver of lateral support. The medial canthal division of the inferior palpebral orbicularis muscle is primarily innervated by the buccal terminal branches responsible for palpebral position, tone, and blinking, and

the extracanthal division responsible for the voluntary closure of the palpebral fissure is innervated by the zygomatic terminal branches.

Consequently, an orbicular myotomy should protect the segment closest to the medial canthus. Despite advances in anatomical studies and blepharoplasty techniques, the ideal access to the procedures remains controversial [25]. To date, no study has evaluated possible structural alterations of the pretarsal muscles in patients who underwent classical transmuscular blepharoplasty via cutaneous access.

The objective of this study is to highlight the histomorphological changes in the pretarsal muscle complex in a group of patients undergoing transcutaneous lower blepharoplasty with myotomy of the orbicularis and another group of patients undergoing transconjunctival blepharoplasty without myotomy.

The unique opportunity to perform histological analyses of the orbicularis oculi muscle after transcutaneous and transconjunctival lower blepharoplasty arose with the spontaneous return of 32 patients desiring minor aesthetic adjustments such as excess skin and residual fat pads. In this group, 12 had undergone blepharoplasty with myotomy and 20 without. The remaining 236 patients were observed for a period of 18 months post-op after which contact was infrequent or none. During this post-op phase, surgical results were satisfactory and patients were content. It should be noted that any alteration following myotomy is only manifest later than this follow-up (2–3 years).

Methods

Between January 2013 and December 2015, 268 patients underwent blepharoplasty performed by a single surgeon: 112 (41.7%) submitted to transcutaneous lower blepharoplasty with a skin–muscle flap, resection of the fat bags and skin, and a lateral support procedure. The muscle incisions were performed at a proper distance from the medial palpebral corner, and straight Steven’s scissors were used to dissect the orbicularis muscle (Fig. 1); 156 (58.2%) submitted to lower transconjunctival blepharoplasty with retroseptal access, resection of the fat bags and pinched skin, and a lateral support procedure (Fig. 2a, b). Patients who underwent previous eyelid surgeries as well as those with Graves’ disease or autoimmune disorders were not included in the study.

Of the 112 patients who underwent transcutaneous blepharoplasty with myotomy of the lower orbicularis muscle, 12 (10.7%) required reoperation 8–14 months later to treat residual lateral palpebral fat bags, slight excess skin, and repeat canthopexy. Of the 156 patients who submitted to transconjunctival inferior blepharoplasty (without myotomy), 20 (12.8%) required reoperation 7–12 months after

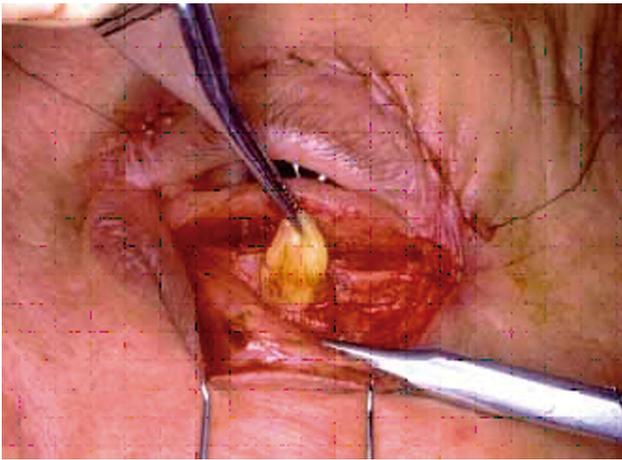


Fig. 1 Transcutaneous blepharoplasty with myotomy for resection of the retroseptal fat bags

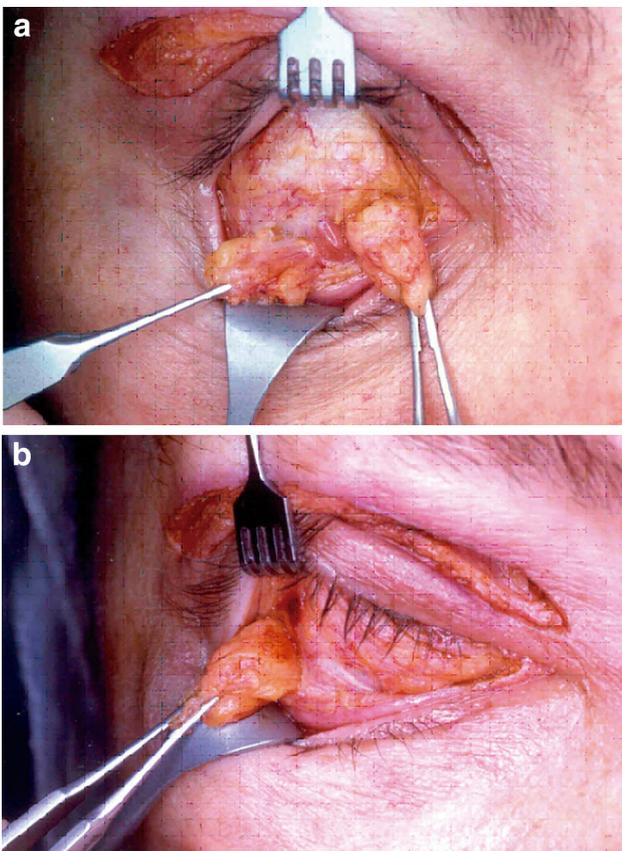


Fig. 2 **a** Transconjunctival blepharoplasty without myotomy of the orbicularis (dissection of the medial and central pouches). **b** Transconjunctival blepharoplasty without myotomy of the orbicularis (dissection of the lateral pouch)

resection of the residual eyelid fat bags and slight excess skin (pinch technique). Of the 32 re-operated patients, 25-mm-long and 3-mm-wide strips of pretarsal muscle were collected.

The samples were fixed in 4% formaldehyde and embedded in paraffin. Five-micrometer-thick slices were created and stained by the following histological techniques: hematoxylin and eosin to characterize tissue integrity, Masson's trichrome for connective tissue quantification, and picrosirius for collagen-type characterization. Immunohistochemical techniques were used to mark the fibers of the elastic system, anti-elastin (1:100; #AB21610; Abcam, Cambridge, MA, USA); blood vessels, anti-CD31 (1:35; polyclonal #ab28364; Abcam), and anti-tubulin β III (1:100; monoclonal #ab78078; Abcam) to characterize the nerves. Fiber diameters were measured in transverse muscle sections of the muscle. Fifty fibers were analyzed per patient in several sites of the muscle strip.

The different analyzed systems were observed and quantified at different amplifications using a microscope (BX51; Olympus, Tokyo, Japan) coupled to a video camera (DP71; Olympus).

Quantitative Analysis

The quantitative analysis was performed using ImageJ[®] 1.47t software (Image Processing and Analysis in Java) to determine the area density of the connective tissue and elastic fibers, nerves, blood vessels, and muscle fiber diameters. Intergroup differences were evaluated by the unpaired Student's *t* test. The results are expressed as mean \pm standard deviation with values of $p < 0.05$ being considered statistically significant. All analyses were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA).

Results

Changes in collagen and nerves were statistically significant (Table 1 and Figs. 3a–d, 5a, b, 6a, b). In addition, the qualitative analysis with picrosirius clearly shows that in patients with myotomy the collagen was predominantly type III (Fig. 4a, b).

Changes in nerve cells were significantly different (Table 1; Figs. 5a, b, 6a, b).

Elastic fibers and blood vessels did not present statistical difference (Table 1; Fig. 7a, b).

Discussion

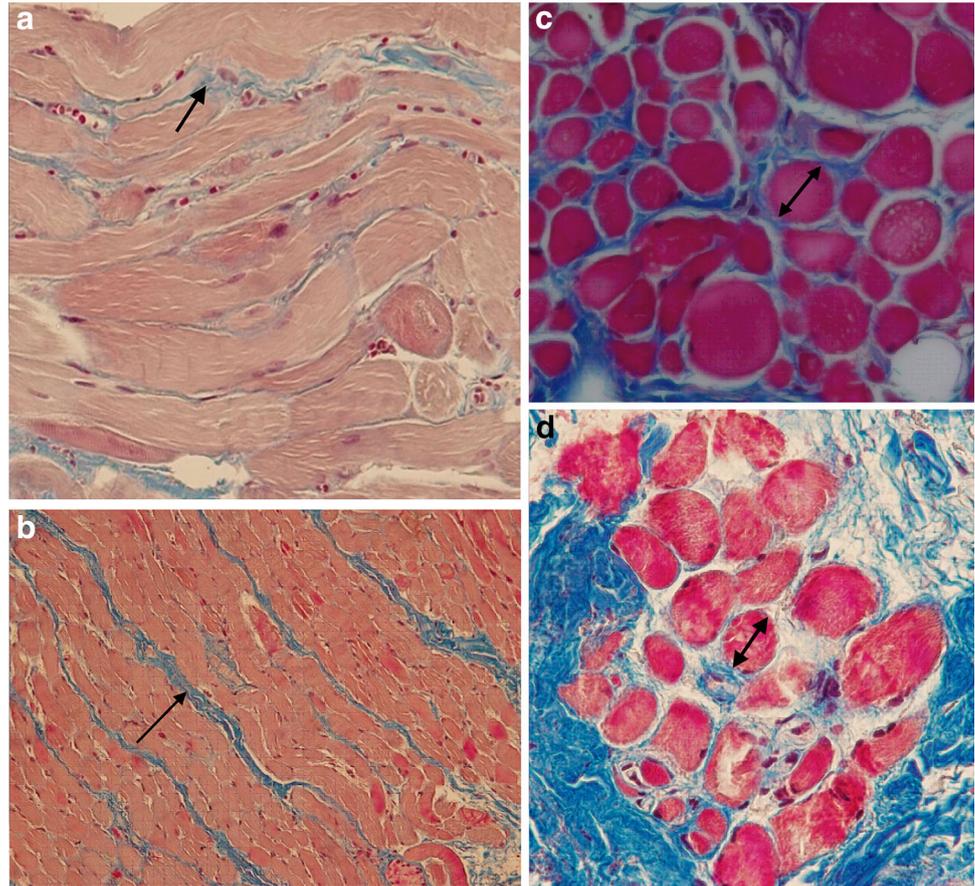
Recent neuroanatomical studies [18, 19, 21] have shown diffuse motor innervation of the lower orbicularis oculi muscle with the contribution of multiple branches of the facial nerve, including the zygomatic and the buccal branches. The lower orbicularis oculi muscle has an

Table 1 Statistical analysis results

	Collagen*, %	Elastin, %	Vessels, %	Nerves*, %	Fiber diameter (μm)
Without myotomy	9.46 \pm 0.41	7.03 \pm 0.62	4.25 \pm 0.06	5.2 \pm 0.55	62 \pm 8.0
With myotomy	19.02 \pm 1.66	7.88 \pm 1.10	5.13 \pm 0.70	2.9 \pm 0.48	49 \pm 6.5

*Significant difference ($p < 0.05$)

Fig. 3 Photomicrograph showing the collagen between the detrusor muscle fibers **a** without myotomy and **b** with myotomy. The arrow indicates the collagen. Masson's trichrome. Final magnification of $\times 200$. Photomicrograph of transversal section of the orbicularis muscle **c** without myotomy and **d** with myotomy. Only the fibers with almost transversal sections were measured. The black line indicates how the diameter was measured. Masson's trichrome. Final magnification of $\times 200$



extremely elaborate innervation conferring special anatomical aspects that makes it completely different from other skeletal muscles [26]. Histological analyses performed in two groups of patients submitted to transcutaneous lower blepharoplasty involving sectioning the lower orbicularis oculi muscle and transconjunctival and preserving the integrity of the orbicularis muscle showed sharp differences in the pretarsal muscle complex as well-defined changes in the connective tissue, elastic system, blood vessels, nerves, and muscle fiber diameters.

The 44% decrease in innervation between the groups with and without myotomy supports the idea of preserving the integrity of the complex motor innervation of the orbicularis oculi muscle during lower blepharoplasties as a fundamental factor in the maintenance of its important and delicate functions. The decrease in the amount of nervous

tissue after myotomy can justify postoperative complications such as malposition of the lower eyelid.

Cumming et al. [27] showed a mean 12% collagen content in the mass of striated muscles and stated that any excess of connective tissue should be considered pathological. Our study showed an increase of approximately 100% of collagen among muscle fibers after myotomy, characterizing a possible malfunction of these fibers subjected to myotomy during blepharoplasty.

There was virtually no intergroup difference in relation to the fibers of the elastic system, showing that these fibers were not affected.

Although the difference between the diameters of the fibers with versus without myotomy was insignificant, a 21% decrease in the diameter of the muscle fibers in the

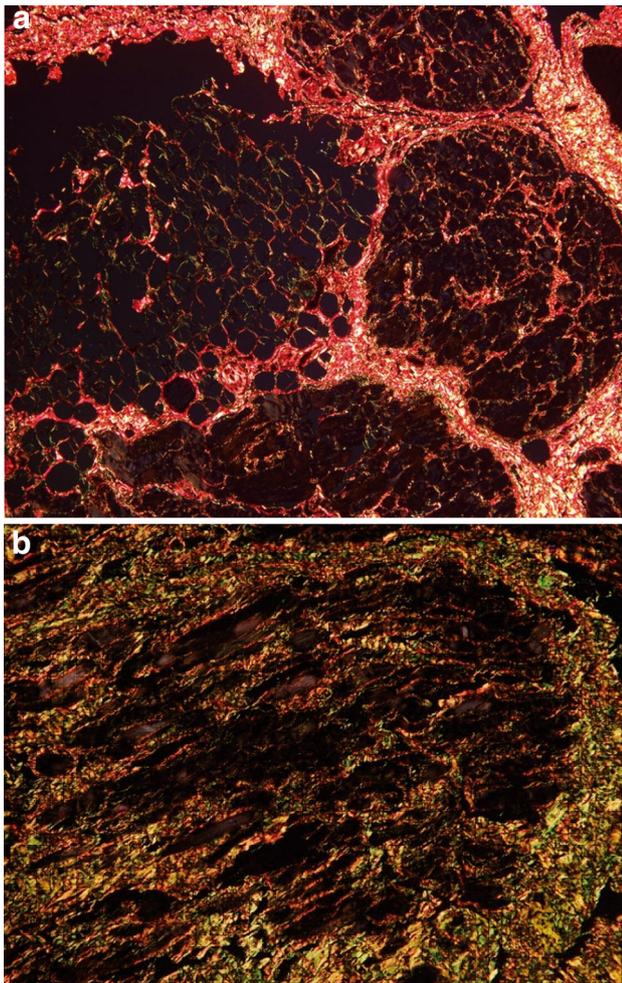


Fig. 4 Qualitative analysis of collagen fibers in the orbicularis muscle **a** without myotomy and with myotomy (**b**). The predominance of red fibers is clearly shown in the muscle without myotomy. In the collagen of muscle with myotomy the fibers presented a greenish color. Picosirius red under polarized light. Final magnification of $\times 200$

group with myotomy corroborates the idea that denervation caused by myotomy led to atrophy of the studied muscles.

According to Stuzin [28], the more common late problems in transcutaneous lower blepharoplasty include denervation of the pretarsal portion of the of the orbicularis oculi with progressive loss of tone. Although many important procedures to maintain eyelid position such as canthoplasty and canthopexy have been described and can minimize the effects of post-myotomy denervation [9], complete preservation of the orbicularis oculi muscle during blepharoplasty can offer a long-term functional advantage for this muscle.

For the purpose of the present study, predominantly longitudinal sections were obtained. The number of fibers cut transversely was small, so the statistical analysis of the fiber diameters is a less reliable difference as more than

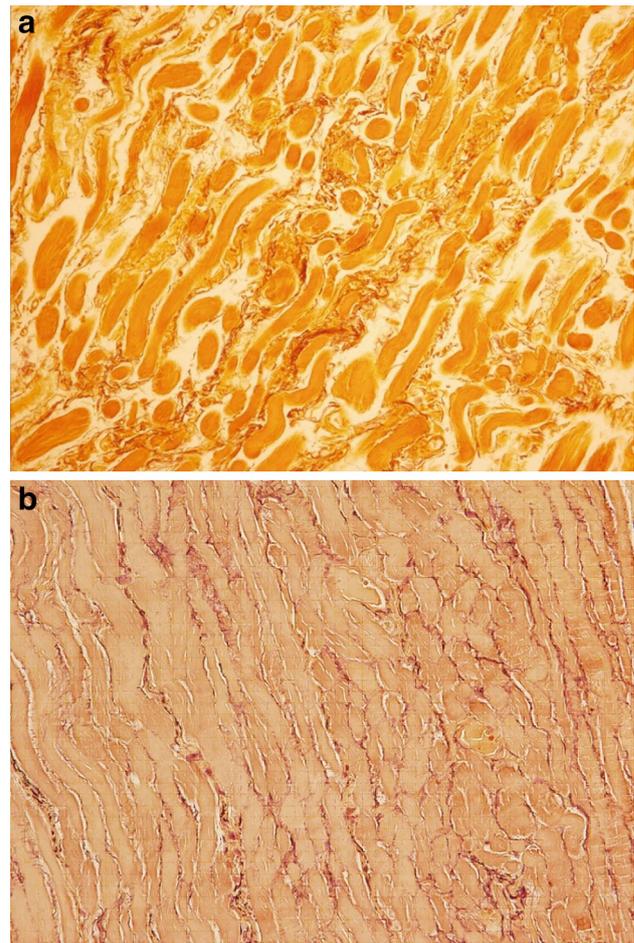


Fig. 5 Photomicrographs illustrating the distribution of elastic fibers in the orbicularis muscle. The figures show that the amounts of fibers are similar in patients with (**a**) or without (**b**) myotomy. Weigert method for elastic fibers, with previous oxidation. Final magnification of $\times 200$

20% indicates a trend that could be statistically significant if a large number of fibers had been analyzed.

The results obtained here show that surgeries with myotomy caused significant changes in collagen, nerves, and diameter of the fibers of the orbicularis oculi muscle. These observations strongly suggest the use of techniques that preserve the integrity of the orbicularis oculi muscle during lower blepharoplasty surgery.

Conclusions

Our histological findings showed that surgeries with myotomy caused significant changes in collagen, nerves, and muscle fiber diameter. These findings suggest that techniques for lower blepharoplasty that spare the integrity of the muscle, namely the transconjunctival approach, are the preferable options. In cases in which there is skin

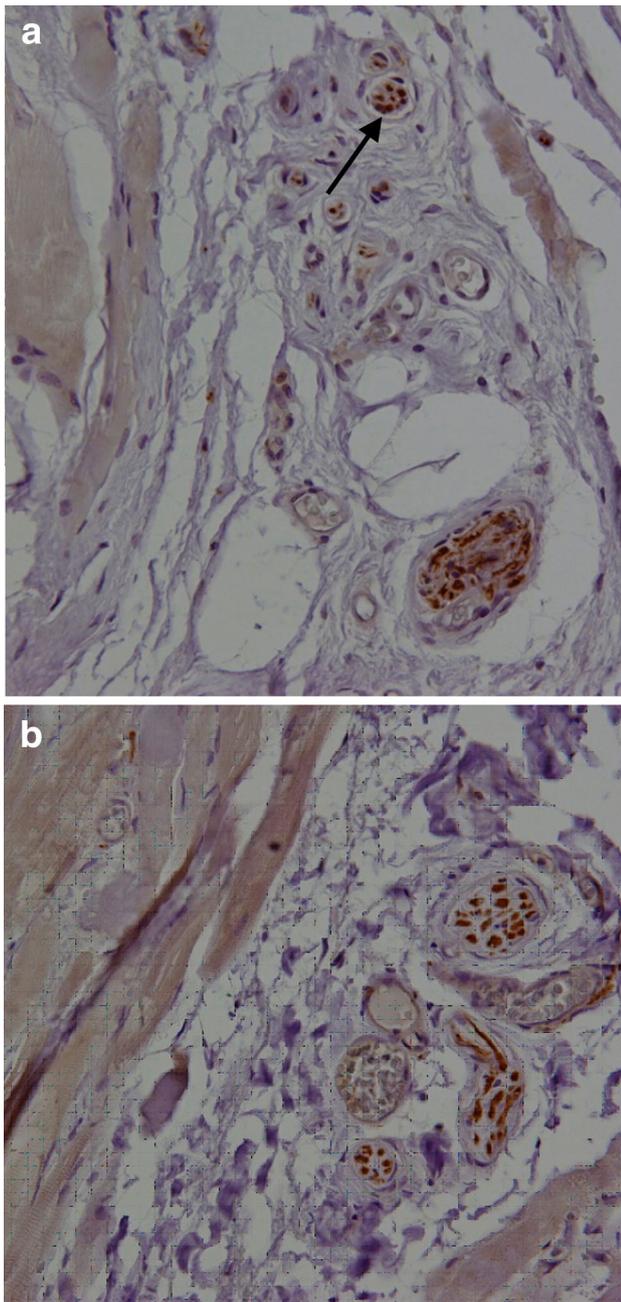


Fig. 6 Photomicrographs illustrating the distribution of tubulin positive in the orbicularis muscle. The immunolabeling showed that the amount of nerves in patients with (a) or without myotomy (b). The arrow indicates the vessels. Final magnification of $\times 200$

excess and fatty pads, we suggest that the fat pads be removed through the transconjunctival access and the skin removed by way of a pinch technique or skin flap. The double access allows for the treatment of fat pads and excess also while preserving the muscle. Canthoplasty or canthopexy may be an additional resource.

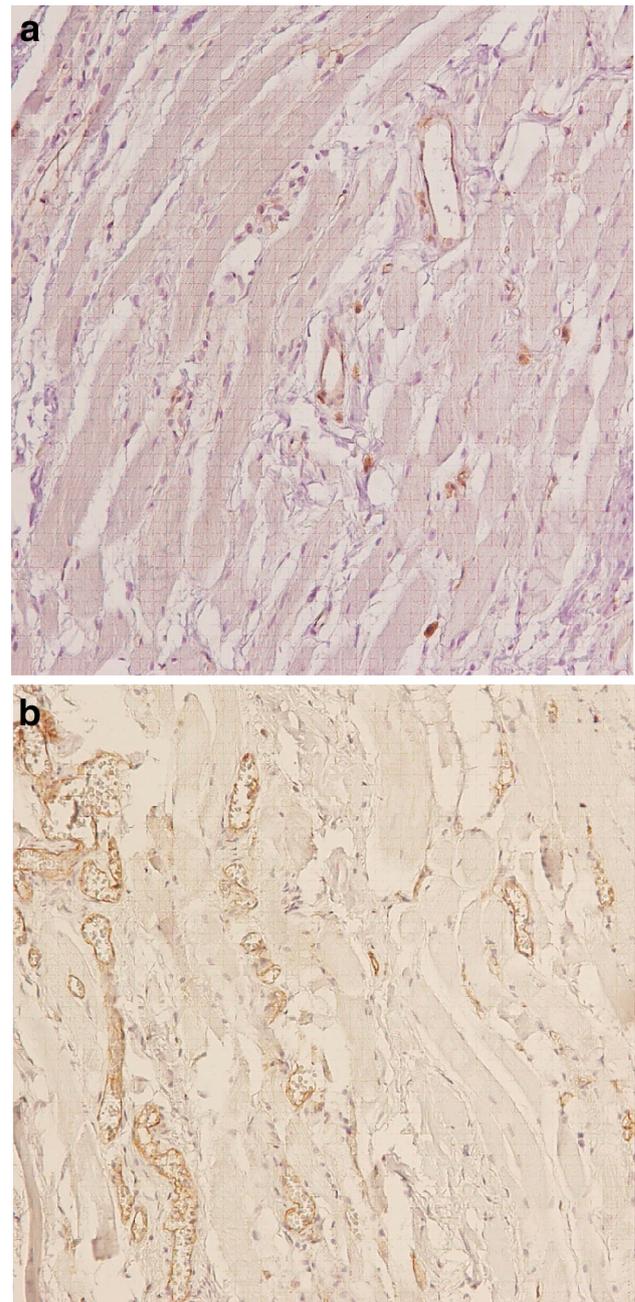


Fig. 7 Photomicrographs illustrating the distribution of CD31 positive in the orbicularis muscle. The immunolabeling showed that the amounts of blood vessels are similar in patients with (a) or without (b) myotomy. Final magnification of $\times 200$

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest to disclose.

Ethical Approval All surgical procedures were performed in the author senior private practice and were in accordance with the ethical standards of the institutional and for national research committee and

the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent All persons involved in this study have given informed consent to include muscle sample for scientific or education purpose and for publication of the results of this study.

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