Enhancing the Growth of Natural Eyelashes: The Mechanism of Bimatoprost-Induced Eyelash Growth

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BACKGROUND Many women desire prominent eyelashes. In December 2008, bimatoprost ophthalmic solution 0.03% was approved for the treatment of hypotrichosis of the eyelashes in the United States.

OBJECTIVE To review eyelash physiology and the proposed mechanisms by which the topical prostamide product bimatoprost enhances eyelash growth.

METHODS AND MATERIALS Clinical and preclinical studies pertaining to the efficacy, safety, and mechanisms of action of bimatoprost are presented.

RESULTS Treatment with bimatoprost increases the percentage of eyelash follicles in anagen at any one time. This probably accounts for its ability to lengthen lashes. Bimatoprost-induced stimulation of melanogenesis appears to result in darker lashes and, at the same time, appears to increase the size of the dermal papilla and hair bulb, affecting lash thickness and fullness. Such effects, largely demonstrated in animal studies, are consistent with the results of a recent Food and Drug Administration phase III clinical trial. The favorable safety profile of bimatoprost in human subjects is probably secondary to the limited exposure of ocular tissues resulting from topical application at the base of the upper lashes.

CONCLUSION By influencing the eyelash hair cycle and follicles, bimatoprost ophthalmic solution 0.03% is a safe and effective means of enhancing eyelash growth.

Dr. Cohen has served as a consultant and clinical trial participant for Allergan, Inc.

The eyes have long been recognized as an important facet of physical beauty.\(^1,2\) Male and female observers have associated enhanced appearance of the eyes of women with significantly greater attractiveness.\(^3\) Beautiful eyes are also associated with social advantages.\(^1\) Long and thick or full eyelashes are a symbol of beauty and femininity in many cultures, whereas the loss of eyelashes has been associated with a loss of attractiveness and psychosocial distress.\(^4-6\) Women often consider longer, thicker, fuller eyelashes to be desirable, and greater growth of one’s eyelashes has been described as having a positive psychological effect.\(^7,8\) For thousands of years, women have employed techniques to enhance the prominence of their eyelashes.\(^9\)

Eyelashes are more than purely aesthetic in nature. By defending the eye against debris and triggering the blink reflex, they serve a protective function against airborne particles.\(^10-12\) In addition to normal variation in eyelash appearance between individuals, some patients experience the loss of previously normal eyelashes, a condition termed madarosis or milphosis.\(^12\) Madarosis can be the result of trauma, endocrine disease (e.g., hypothyroidism), drugs (e.g., antimetabolites), radiation, or rarely, infection (e.g., leprosy); if the hair follicles remain, the normal growth of eyelashes often resumes when the underlying cause of disease is treated. Patients suffering from madarosis may benefit from treatments aimed at improving the appearance of their eyelashes.
Although once limited, today, women have several options for enhancing the appearance or prominence of their eyelashes. Available for centuries, mascara uses waxes, pigments, and resins to lengthen, thicken, and darken eyelashes. The effects of mascara are temporary and subject to smudging. Artificial eyelashes or eyelash extensions can stay in place from several days to several weeks and offer women another option for improving the appearance of their eyelashes. Methacrylate-based adhesives are used to hold the lashes in place and are typically removed using solvents; the adhesives and solvents can both cause allergic reactions in some patients. An invasive permanent method of increasing the prominence of eyelashes is transplantation, which transfers hair follicles from the scalp onto the margins of the eyelid. This result is eyelashes that have qualities of scalp hair and require regular trimming and curling. Most recently, a new exciting and simple option for enhanced eyelashes became available with Food and Drug Administration (FDA) approval of topical bimatoprost ophthalmic solution 0.03% (Latisse, Allergan, Inc., Irvine, CA) for the treatment of hypotrichosis of the eyelashes by increasing their growth and enhancing length, thickness or fullness, and darkness.

This article will review the physiology underlying normal eyelash growth and the proposed mechanisms of action by which the prostamide bimatoprost enhances the growth of eyelashes. We will review preclinical and clinical data regarding the safety of bimatoprost and its effects on eyelash growth.

Eyelash Properties and Hair Cycle

On each upper eyelid, eyelashes are arranged in two to three rows for a total of approximately 100 to 150 lashes. Upper eyelashes are more numerous and longer than lower lashes and, unlike lower lashes, curve upward. Eyelashes are terminal hairs, which, in contrast to vellus hairs, are longer, medullated, and pigmented (Figure 1). Unlike other hairs, eyelashes are devoid of arrectores pilorum muscles. In a recent study, Na and colleagues described human eyelashes as being approximately 9 mm in length, with 7 mm of that length extending beyond the skin. In the same study, eyelashes exhibited a growth rate of approximately 0.15 mm/day. Of all human hairs, eyelashes are the widest and most pigmented. Eyelashes do not typically turn grey with age.

The growth of eyelashes is cyclical and, like all hairs, can be divided into three main stages: anagen, catagen, and telogen (Figure 2). In humans, unlike many mammals, the hair cycle is asynchronous...
Nous, such that some hair follicles are growing (in anagen) while others are dormant (in telogen).\(^{20,23}\)
The normal eyelash cycle is variable and lasts approximately 5 to 12 months.\(^{19–21}\)
The growth phase of the eyelash follicle, anagen, is approximately 1 to 2 months long. It is the duration of anagen that largely determines hair length.\(^{10}\)
During anagen, melanocytes located over the apex of the dermal papilla engage in melanogenesis and the subsequent transfer of pigment to the medullary and cortical cells of the follicle.\(^{20}\)

After completing anagen, hair follicles enter the transition phase, catagen. During catagen, the epithelial elements of the follicle undergo apoptosis, or programmed cell death. In eyelashes, this phase takes approximately 15 days.\(^{20}\)
From catagen, the eyelash follicle enters telogen, the resting phase, which can last from 4 to 9 months.\(^{19–21}\)
The “old” hair is expelled from the follicle during exogen, which occurs in the transition from telogen to anagen.\(^{10,24}\)

**Eyelashes Versus Scalp Hair**

Although the basic hair cycle is similar between eyelashes and scalp hair, a number of distinct differences exist (Table 1).\(^{19–24}\)
Such differences alter growth patterns. At any given time, approximately 41% of upper eyelid eyelash follicles are active,
compared with approximately 84% of scalp follicles.\textsuperscript{19,20} Conversely, approximately 50% of eyelash follicles are in telogen compared with 5% to 15% of follicles on the scalp.\textsuperscript{19,20,23,24} Scalp hair has a much longer anagen phase and a shorter telogen phase than eyelashes. Scalp hair follicles can grow (remain in anagen) for as long as 8 years, and scalp hairs grow at a rate of 0.3 to 0.4 mm per day.\textsuperscript{20,22–24}

| TABLE 1. Comparison of Scalp Hair With Upper Eyelashes\textsuperscript{19–24} |
|-----------------------------|-----------------------------|
| Scalp Hair                  | Upper Eyelashes             |
| Hair cycle duration         | > 8 years                   | 5–12 months                |
| Anagen duration             | 6–8 years                   | 1–2 months                 |
| Percentage of follicles in  | 5–15%                       | 50%                        |
| telogen                     |                             |                            |
| Androgen sensitivity        | Yes                         | No                         |
| Average growth rate, mm/day | 0.30–0.40                   | 0.15                       |
| Number of follicles         | ~ 100,000                   | 100 to 150 per eyelid      |

The differences between scalp hair and eyelashes have important implications for the development and use of hair growth drugs on these hairs. The characteristics of the eyelash growth cycle suggest that treatments that initiate or prolong anagen may have more immediate, visible effects on eyelashes than on the nonbalding scalp.

**Bimatoprost—Eyelash Growth in Glaucoma Trials**

Bimatoprost is a synthetic prostamide $F_{2\alpha}$ analog.\textsuperscript{29} The prostamides and their structural analogs are structurally, pharmacologically, and functionally distinct from prostaglandins and prostaglandin analogs (Figure 3).\textsuperscript{29–31} Bimatoprost ophthalmic solution 0.03% (Lumigan, Allergan, Inc., Irvine, CA) was approved in 2001 for the reduction of high intraocular pressure (IOP) in patients with open-angle glaucoma or ocular hypertension.\textsuperscript{32} It is considered the most efficacious antiglaucoma drug available,\textsuperscript{29} and its safety and effectiveness as an IOP-lowering agent has been established in clinical trials lasting up to 4 years.\textsuperscript{33,34}

Bimatoprost exerts its effects by stimulating the prostamide receptor, which is pharmacologically distinct from F prostanoid (FP) receptors.\textsuperscript{29,34} Although the existence of the prostamide receptor, an entity distinct from the FP receptor, has been established via multiple lines of evidence,\textsuperscript{29,34–36} it has been hypothesized that FP and prostamide receptors may be messenger ribonucleic acid–splicing variants of the same gene.\textsuperscript{29} Although prostaglandin receptors involved in the development and regrowth of the hair follicle have been identified throughout the hair follicle, particularly in the dermal papilla outer root sheath,\textsuperscript{37,38} it is unknown whether bimatoprost exerts effects on receptors in these locations. The ability of bimatoprost to affect eyelash growth and appearance was first detected in clinical trials of the drug as an ocular antihypertensive agent. In these
trials, eyelash growth was recorded as an adverse event with use of this agent as an eyedrop. In a pair of 1-year, multicenter, randomized, double-masked, parallel-group, active-controlled trials, 42.6% of patients treated with bimatoprost once daily (1 drop instilled into each eye) experienced eyelash growth.\textsuperscript{39} Other than eyelash growth, the most common adverse events reported when bimatoprost is administered as an eyedrop (for the treatment of ocular hypertension) are conjunctival hyperemia, eye pruritus, eye dryness, a burning sensation in the eye, eyelid pigmentation, foreign body sensation, eye pain, and visual disturbance.\textsuperscript{32,33,39} The skin pigmentation observed with bimatoprost used as an eyedrop can become noticeable after several months of use and is reversible upon discontinuation of the drug.\textsuperscript{7,40} Such changes appear to be preventable by minimizing contact with the skin.\textsuperscript{41} It has been suggested that such skin changes, if targeted to specific locations, may be desirable as a semipermanent cosmetic.\textsuperscript{29,34}

When reported in clinical trials for high IOP, the changes in eyelash growth were not quantified, making it difficult to compare the effects of bimatoprost with placebo or an active comparator. Eyelash changes have also been reported for other drugs used to treat glaucoma, including the prostaglandin analogs latanoprost and travoprost.\textsuperscript{7,20,40} The safety and efficacy of these agents for the treatment of hypotrichosis of the eyelashes have not been evaluated in double-blind, placebo-controlled, randomized trials and therefore are largely excluded from the present discussion. Although eyelash growth was not further characterized, in a 3-month head-to-head trial assessing the comparative efficacy of once-daily bimatoprost 0.03% and latanoprost 0.005% for the treatment of ocular hypertension, eyelash growth was significantly more common in patients treated with bimatoprost than with latanoprost (12.6% vs 4.4%; \(p = .03\)).\textsuperscript{42} How such differences correlate to the relative effects of these drugs when applied topically to the base of the upper lashes is not known.

Bimatoprost—Mechanism of Action

A series of recent animal (mouse) studies have revealed that treatment with bimatoprost results in multiple changes to the hair cycle of eyelashes (Figure 4) (unpublished data). A 2-week course of bimatoprost resulted in a greater proportion of follicles in the anagen phase of the hair cycle. A concomitant decrease in the percentage of follicles in telogen was observed, suggesting that bimatoprost stimulated the transition from telogen to anagen and that bimatoprost treatment was associated with prolonged duration of anagen. Such changes in the length of anagen probably manifest as observable increases in eyelash length associated with treatment.
The influence of bimatoprost on eyelash thickness or fullness has also been demonstrated in an animal model (unpublished data). In mice, treatment with bimatoprost resulted in significant increases in the thickness or fullness of medium (~450 μm) and short (~250 μm) eyelashes. These eyelashes became approximately 20% thicker or fuller than untreated ones. Significant increases in thickness were not observed in long (~2500 μm) eyelashes. Furthermore, bimatoprost treatment was associated with larger dermal papilla and hair bulb diameters in early-anagen follicles. For example, the mean hair bulb diameter increased more than 29% (vs vehicle) in early anagen follicles. Such differences were not observed in follicles in late anagen.

The ability of bimatoprost to increase the darkness of eyelashes probably results from greater melanogenesis. Kapur and colleagues demonstrated that treatment with bimatoprost appears to result in an increase in melanin granules without concomitant proliferation of melanocytes, cellular atypia, or evidence of an inflammatory reaction (as would be expected if the mechanism of action was similar to that observed with irritant contact dermatitis). It has been hypothesized that a possible mechanism for greater melanogenesis is stimulation of the tyrosinase enzyme (via direct effect, greater transcription of the gene, or both), the key rate-limiting enzyme in melanin synthesis.

The aforementioned studies suggest that bimatoprost can increase the length, thickness or fullness, and darkness of eyelashes, all traits that may be associated with greater prominence and overall appearance of eyelashes. As anticipated, animal studies confirm that bimatoprost treatment does not affect the number of eyelash follicles (unpublished data). In a 4-week mouse study, 2 weeks of treatment with bimatoprost resulted in a significantly greater (~20%) number of eyelashes than was observed on eyes in the control group. It is likely that the greater number of visible hairs was a result of new lashes forming more quickly (transition from telogen to anagen) and existing lashes remaining longer (exogen being delayed) in the same follicle as the new hair. Bimatoprost may also be capable of causing vellus hairs to become visibly apparent.

**Bimatoprost for Eyelash Growth—Clinical Trial**

To assess the safety and efficacy of bimatoprost 0.03% solution once daily in increasing overall eyelash prominence after topical administration to the upper eyelid margins, a 5-month, phase 3, multicenter, randomized, double-masked, vehicle-controlled study was conducted. As reported by Smith and colleagues, 278 healthy adults with minimal or moderate eyelash prominence as assessed using an investigator-rated scale (the Global Eyelash Assessment (GEA)) enrolled in the trial; 137 received bimatoprost 0.03%, and 141 received vehicle. Subjects were instructed to place 1 drop of study drug onto a disposable, single-use-per-eye applicator and apply it to each upper eyelid margin in the evening for 4 months. The primary efficacy measure was the proportion of subjects demonstrating an improvement in overall eyelash prominence as assessed using the GEA, an investigator-rated, reliable, and reproducible 4-point scale. GEA scores of 1, 2, 3, and 4 indicate minimal, moderate, marked, and very marked eyelash prominence, respectively. From week 8 through the week-20 posttreatment visit, a
significantly greater percentage of subjects demonstrated improvements in eyelash prominence than those treated with vehicle (Figure 5).

In the bimatoprost for eyelash growth trial, efficacy was also assessed using digital photographs and subsequent digital image analysis that measured eyelash length, thickness or fullness, and intensity (darkness). Bimatoprost was associated with significantly greater improvement than vehicle in all three eyelash traits from week 8 onward. At week 16, subjects in the bimatoprost group had a mean change in eyelash length of 1.39 mm, whereas the vehicle group exhibited a mean change of 0.11 mm ($p<.001$). This correlated to a 24% increase in the bimatoprost-treated group, compared with 2% for vehicle. Mean increases in eyelash thickness or fullness at week 16 were 0.71 mm$^2$ for subjects treated with bimatoprost and 0.06 mm$^2$ for subjects receiving vehicle ($p<.001$), correlating to a 106% increase in the bimatoprost-treated group and a 12% increase in the vehicle-treated group. At week 16, the bimatoprost-treated cohort exhibited an 18% increase in eyelash darkness, compared with 3% for vehicle ($p<.001$).

As highlighted in Figure 5, by week 8, 50.4% of the bimatoprost-treated subjects demonstrated changes in their eyelashes constituting a 1-grade increase in their GEA score, and this percentage continued to increase through the end of the study. Many of the subjects not meeting GEA criteria for greater eyelash prominence still exhibited considerable positive changes in the appearance of their eyelashes as assessed using digital image analysis. As early as week 8, for instance, the bimatoprost-treated group demonstrated median increases in eyelash length, thickness or fullness, and darkness of 0.6 mm, 18.25%, and 10.32%, respectively. A post hoc analysis demonstrated that, at week 16, the 21.9% of subjects receiving bimatoprost who had not achieved at least a 1-grade increase in GEA score exhibited greater mean increases in the digitally assessed qualities of eyelash length, thickness or fullness, and darkness than the subjects receiving vehicle.

In addition to the quantitative assessments described above, the pivotal trial of bimatoprost for eyelash growth used a series of four patient-reported outcome questionnaires (unpublished data). Treatment with bimatoprost was associated with significantly greater increases in patient satisfaction on all items on the primary patient-reported outcome questionnaire, a 23-item static assessment of subjects’ perspectives collected at every visit (Table 2) (data on file, Allergan, Inc.). Bimatoprost-treated subjects reported statistically significantly higher levels of satisfaction with their eyelashes in terms of the physical attributes of their eyelashes, the subjective attributes of their eyelashes (satisfaction with eyelashes as they relate to feelings of confidence, professionalism, and attractiveness), and the daily routine of making their eyelashes presentable than vehicle-treated subjects.

### Bimatoprost for Eyelash Growth—Safety

Overall, bimatoprost applied to the dermal margin of the eyelid was associated with a particularly favorable safety profile in the pivotal trial (unpublished data). Conjunctival hyperemia was the only...
adverse event reported significantly more often in subjects receiving bimatoprost \((n = 5)\) than those receiving vehicle \((n = 0)\). All cases of conjunctival hyperemia in the bimatoprost-treated subjects were transient and resolved before the end of the study despite continued application of the product. When applied to the eyelid, the incidence of conjunctival hyperemia \((3.6\%)\) associated with bimatoprost was less than one-tenth of that observed in a 3-month study when bimatoprost was administered as an eyedrop for glaucoma or ocular hypertension.\(^{48}\) Although statistically significant, the effects of bimatoprost for eyelash growth on IOP were not thought to be clinically relevant (unpublished data). Another difference in the observed safety profile of bimatoprost when applied to the eyelid as opposed to used as an eyedrop was the absence of reports of iridal pigmentation.\(^{17}\) Pigmentation of the iris is a rare, but potentially permanent, adverse event observed in some patients receiving bimatoprost eyedrops for the treatment of glaucoma and is thought to occur within the first year \((3 \text{ to } 12 \text{ months})\) of therapy,\(^{33}\) with an incidence of 1.5%.\(^{39}\) Iris pigmentation changes are associated with the ability of the agent to stimulate melanogenesis and are aesthetic in nature, not appearing to present any safety concerns, such as melanocyte proliferation.\(^{45}\)

It is possible that less exposure of the ocular tissues to bimatoprost when applied topically accounts for the differences in the safety profile of the drug. For instance, it has been estimated that a single application of bimatoprost 0.03% to the upper eyelid margin using the supplied applicator delivers approximately 5% of the dose \((\text{by weight})\) administered in an eyedrop for the treatment of glaucoma (unpublished data). Low ocular tissue exposure to a solution applied topically to the eyelid margin was demonstrated using application of the ophthalmic dye, lissamine green (Figure 6). Additionally, secondary to the barrier formed by the skin, absorption of active drug from the cutaneous surface into ocular tissues is expected to be minimal.

<table>
<thead>
<tr>
<th>TABLE 2. Items Assessed According to Patient-Reported Outcome Questionnaire Administered During Bimatoprost for Eyelash Growth Pivotal Clinical Trial</th>
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<tbody>
<tr>
<td>Satisfaction with length of eyelashes</td>
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<tr>
<td>Satisfaction with fullness or thickness of eyelashes</td>
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<tr>
<td>Satisfaction with darkness of eyelashes</td>
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<tr>
<td>Satisfaction with eyelashes, overall</td>
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<tr>
<td>Without mascara, eyelashes look full</td>
</tr>
<tr>
<td>Eyelash length rating</td>
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<tr>
<td>Eyelash fullness or thickness rating</td>
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<tr>
<td>Eyelash color rating</td>
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<tr>
<td>Amount of time spent applying mascara is bothersome</td>
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<tr>
<td>Amount of time spent removing mascara is bothersome</td>
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<tr>
<td>Hassle to spend time making eyelashes presentable</td>
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<tr>
<td>Able to go out in public without mascara</td>
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<tr>
<td>Worry about mascara smearing</td>
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<tr>
<td>Eyes look tired*</td>
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<tr>
<td>Eyelashes look naturally attractive*</td>
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<tr>
<td>Feel confident in looks*</td>
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<tr>
<td>Feel confident going out in public</td>
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<tr>
<td>Feel confident about professional appearance*</td>
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<td>Feel attractive*</td>
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<tr>
<td>Eyelashes look healthy*</td>
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<tr>
<td>Eyes look vibrant*</td>
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<tr>
<td>Frequency of compliments from others about eyelashes</td>
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<tr>
<td>Feel beautiful*</td>
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</tbody>
</table>

*Questions were asked in the context of appearance without the use of mascara.

Figure 6. Application of lissamine green dye as an eyedrop or applied to the upper eyelid margin. Note the greater exposure of the conjunctiva, cornea, and periocular skin to the dye when administered as an eyedrop than when dermally applied using an applicator.
Conclusions

With many women wishing to improve the appearance of their eyelashes, bimatoprost ophthalmic solution 0.03% provides a new, safe, and effective option for growing natural eyelashes, making them longer, thicker or fuller, and darker. The ability of bimatoprost to enhance eyelash growth emerged as a serendipitous “side effect” when the drug was instilled as an eyedrop to treat glaucoma. Animal models suggest that bimatoprost stimulates follicles to remain in the anagen phase longer while shortening the telogen phase of the hair cycle. This supports the hypothesis that bimatoprost increases the percentage of eyelashes in anagen and the duration of anagen to result in longer lashes. Bimatoprost also stimulates melanogenesis and enlarges the diameter of the hair shaft, accounting for greater darkness and thickness or fullness of eyelashes, respectively.

The aesthetic effects of bimatoprost were established in a large controlled clinical trial that demonstrated that the topical application of bimatoprost 0.03% to the upper eyelid is associated with significant growth of a subject’s own natural eyelashes and results in longer, thicker or fuller, and darker eyelashes within 4 months of treatment. Subjects in the bimatoprost group also reported significantly greater increases in satisfaction than those in the vehicle group. The trial enrolled healthy adults, and therefore, the ability of bimatoprost to stimulate growth of eyelashes in cases of trauma or alopecia or in patients with other relevant medical conditions has not been fully studied. Future research should evaluate the effects of bimatoprost on these subgroups.

The safety of bimatoprost for the reduction of IOP has been well established in clinical trials up to 4 years in length, as well as extended clinical experience totaling an estimated 9 million patient-years of exposure. Topical administration of bimatoprost 0.03% results in lower exposure to the drug than ocular administration, potentially explaining the low incidence of side effects with the cutaneous method of administration. Taken together, current evidence suggests that topical cutaneous administration of bimatoprost 0.03% to the upper eyelid margin is a safe and effective means of enhancing eyelash growth.

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