Subbasal Nerve Density and Corneal Sensitivity After Laser In Situ Keratomileusis

Femtosecond Laser vs Mechanical Microkeratome

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Objective: To compare changes in subbasal nerve density and corneal sensitivity after laser in situ keratomileusis (LASIK) with the flap created by a femtosecond laser (bladeless) vs a mechanical microkeratome.

Design: In a randomized paired-eye study, 21 patients received myopic LASIK with the flap created by a femtosecond laser in one eye and by a mechanical microkeratome in the fellow eye. Eyes were examined before and at 1, 3, 6, 12, and 36 months after LASIK. Central subbasal nerve density was measured by using confocal microscopy. Corneal mechanical sensitivity was measured by using a gas esthesiometer and was expressed as the ratio of mechanical threshold in eyes that received LASIK to mechanical threshold in concurrent control eyes.

Results: Subbasal nerve density and corneal sensitivity did not differ between methods of flap creation at any examination. Mean (SD) nerve density was decreased at 1 month (bladeless, 974 [2453] µm/mm²; microkeratome, 1308 [2881] µm/mm²) compared with the preoperative examination (bladeless, 10 883 [5083] µm/mm², \(P < .001\); microkeratome, 12 464 [6683] µm/mm², \(P < .001\)) and remained decreased through 12 months (\(P < .001\)). Mechanical threshold ratios did not differ from that at the preoperative examination through 36 months for either LASIK treatment; when all LASIK eyes were combined, the mechanical threshold ratio was transiently higher (decreased sensitivity) at 1 month (1.29 [0.85]) compared with the preoperative examination (0.89 [0.73], \(P = .05\)).

Conclusions: The planar configuration of the femtosecond laser flaps is not associated with faster reinnervation compared with the microkeratome flaps. The prolonged decrease in subbasal nerve density after LASIK is not accompanied by a prolonged decrease in corneal sensitivity.

Trial Registration: clinicaltrials.gov Identifier NCT00350246


ORNEAL NERVES ARE SEVERED AND ABLATED DURING LASER IN SITU KERATOMILEUSIS (LASIK), and regrowth of subbasal nerves is not complete several years after surgery. Denervation can result in a presumed neurotrophic epithelium with ocular surface symptoms and signs. LASIK flaps have traditionally been created with mechanical microkeratomes, but more recently, femtosecond laser technology has emerged as an alternative for flap creation and has been suggested to induce fewer signs of dry eye compared with microkeratomes. The very short duration of pulses delivered by femtosecond lasers enables precise corneal cuts of any geometric configuration. LASIK flaps created with femtosecond lasers are typically planar in contrast to flaps created by mechanical microkeratomes, which are thinner in the center than at the periphery and have tapered edges. The planar configuration of femtosecond laser flaps might allow improved realignment of the flap after LASIK compared with flaps created with mechanical microkeratomes and therefore confer an advantage for reinnervation and the return of corneal sensitivity.

In this randomized paired-eye study, we investigated whether the reinnervation of corneal subbasal nerves was faster after LASIK with a planar flap created by a femtosecond laser compared with LASIK with the flap created by a mechanical microkeratome. We also assessed changes in corneal sensitivity to mechanical stimuli during the first 3 years after surgery.

METHODS

PARTICIPANTS

Twenty-one participants were recruited from patients attending the refractive surgery service at Mayo Clinic. All patients had myopia or myopic astigmatism. This study was approved by the Mayo Clinic Institutional Review Board. All patients provided written informed consent.
opmic astigmatism, were 21 years or older, and were determined to be suitable candidates for LASIK after a rigorous screening examination. The mean (SD) participant age at surgery was 38 (10) years (range, 22-54 years). Twenty myopic, unoperated-on control participants who did not receive LASIK were also recruited and examined concurrently with those receiving LASIK. The mean (SD) age of the controls at their first examination was 38 (9) years (range, 21-31 years). Individuals were excluded if they had any corneal abnormalities; history of ocular disease, trauma, or surgery; or diabetes mellitus or other systemic disease known to affect the eye or if they used oral medications. Systemic medications were permitted unless they were known to affect the cornea or anterior segment. This study complied with requirements of the Health Insurance Portability and Accountability Act and was approved by the Mayo Clinic institutional review board. Informed consent was obtained from all participants after explanation of the nature and possible consequences of the study.

RANDOMIZATION

Participants receiving LASIK were stratified by ocular dominance. One eye of each patient was randomized to receive LASIK with the flap created by a femtosecond laser and the other eye received LASIK with the flap created by a mechanical microkeratome.

LASIK PROCEDURE

Bladeless flap creation was performed with a 15-kHz femtosecond laser (IntraLase FS; IntraLase Corp, Irvine, California). All flaps were created to have a superior hinge with hinge angle of 45° or 55°, an intended thickness of 120 µm, and an intended diameter of 9.0 mm. Raster energy was 2.3 µJ, and side-cut energy was 2.5 µJ. The side-cut angle was 60° or 70° in all cases. Flaps created by the mechanical microkeratome (Hansatome; Bausch & Lomb, Rochester, New York) had a superior hinge with an intended thickness of 180 µm and an intended diameter of 8.5 or 9.5 mm. The stromal bed was ablated with an excimer laser (VISX Star S4; VISX, Santa Ana, California) at a radiant exposure of 160 mJ/cm². Emmetropia was attempted in all cases by using an ablation zone that ranged from 6.5 mm for spherical corrections to 6.5 mm for astigmatic corrections. Postoperative topical medication regimens were identical for each eye and consisted of ciprofloxacin hydrochloride ophthalmic solution, 4 times daily with a taper that extended across 3 weeks.

OUTCOME MEASURES

Patients were examined before LASIK and at 1, 3, 6, 12, and 36 months after surgery. Controls did not receive a surgical intervention and were examined concurrently with the LASIK patients but only at 0, 1, 12, and 36 months. At each examination, subbasal nerve density and mechanical corneal sensitivity were measured. Central flap thickness at 1 month was measured by using confocal microscopy as previously reported.

SUBBASAL NERVE DENSITY

Subbasal nerve density was measured from confocal microscopy images acquired with a ConfoScan 3 confocal microscope (Nidek Technologies, Greensboro, North Carolina) for examinations before June 1, 2005, and with a ConfoScan 4 confocal microscope (Nidek Technologies, Freemont, California) for examinations from June 1, 2005, and afterward. Images were acquired by continuous through-focusing as described in detail previously. Briefly, after anesthetizing the cornea with topical proparacaine hydrochloride, 0.5%, an optical coupling medium (GenTeal Gel; Novartis Ophthalmics, East Hanover, New Jersey) was placed on the tip of the objective and z-ring adapter, and the operator advanced the objective and z-ring until the coupling medium contacted the cornea (the z-ring was available for the ConfoScan 4 confocal microscope only). The objective was aligned to obtain a centered image of the corneal endothelium; the focal plane was then advanced into the anterior chamber before initiating a through-focus examination of the full-thickness cornea from posterior to anterior. The focal plane was automatically reset to the starting position and the full-thickness scan was repeated. Video frames were recorded at 25 frames/s; the step distance between frames was 5 µm. Each cornea was scanned 2 to 4 times.

All images were reviewed, and the best image containing corneal subbasal nerves was manually selected. Randomized images of the nerves were given to a masked observer (K.M.K.), and the subbasal nerves were traced by using a semiautomated image analysis program (Neuro, a plug-in program to ImageJ). The total length of corneal nerves within a central sample area of the image was measured, and nerve density was expressed as the length of corneal nerves per unit area. The same area was 194 × 298 µm (horizontal × vertical) and was selected to minimize use of peripheral regions of the image where nerve visibility was greatly reduced.

CORNEAL SENSITIVITY

Corneal sensitivity was measured by using a gas esthesiometer operated by an observer who was masked to the treatment received by each eye. A mechanical stimulus was provided by a stream of air directed at the center of the cornea through a nozzle with an inside diameter of 0.5 mm, positioned 5 mm from the central cornea. The stimulus flow rate ranged from 25 to 270 mL/min, and the air was warmed to minimize the effects of evaporative cooling on the corneal surface. Stimuli were administered to the central cornea by turning on the airflow for 2 seconds. After each stimulus, the participant reported whether he or she experienced a sensation. A super-threshold stimulus (150 mL/min) was administered first. Flow rate was then reduced to 25 mL/min, a subthreshold rate, and stimuli were then administered at flow rates that increased in steps of 25 mL/min until the participant reported a sensation. The next stimulus was administered at 12 mL/min less than the detected stimulus. If the participant felt this stimulus, we assumed that the threshold was at a flow rate 6 mL/min below this rate; if the patient did not feel it, we assumed that the threshold was 6 mL/min above this rate. A null stimulus (no airflow) was administered at least once during each trial to ensure that participants did not incorrectly respond (false-positive). Measurement of left and right eyes was alternated between stimulus presentations, maintaining a minimum 2-minute interval between stimulus administrations in each eye. If the participant did not feel the initial stimulus at 150 mL/min, the next stimulus was increased to 250 mL/min. If this was felt, the next stimulus was administered at 175 mL/min, and subsequent stimuli were increased in 25 mL/min steps as described.

During the 3-year study, we noticed a significant change in mechanical threshold of control eyes at 36 months compared with previous examinations. As a result, in addition to reporting the absolute mechanical thresholds and comparing absolute mechanical thresholds in LASIK eyes with those in control eyes, we calculated a mechanical threshold ratio, which was the ratio of the mechanical threshold in LASIK eyes to the mean mechanical threshold of control eyes at the concurrent examination. Unlike the LASIK participants, controls were not examined at 3 and...
6 months. Thus, the mean mechanical threshold of control eyes at 1 month was used for comparison with the absolute thresholds at 3 and 6 months to calculate the threshold ratio at 3 and 6 months. A mechanical threshold ratio higher than 1 indicated lower corneal sensitivity of LASIK eyes relative to controls, and a mechanical threshold ratio less than 1 indicated higher corneal sensitivity of LASIK eyes relative to controls.

STATISTICAL ANALYSIS

Differences between eyes (ie, treatments) at each examination and differences between preoperative and postoperative examinations for each treatment were assessed by using 2-tailed paired t tests if the data were distributed normally and Wilcoxon signed rank tests if they were not. Differences between treatments and differences between the postoperative examinations compared with preoperative examinations within each treatment were adjusted for multiple comparisons by using the Bonferroni method. Differences in mechanical threshold after LASIK were also assessed for all eyes, regardless of the method of flap creation, by using generalized estimating equation models to account for possible correlation between fellow eyes of the same participant. Differences between LASIK eyes and controls eyes were also assessed by using generalized estimating equation models. \( P \leq .05 \) was considered statistically significant.

The trial was powered to assess differences in visual acuity and corneal haze between treatments. Corneal nerve density and sensitivity were secondary outcomes, and thus post hoc power analyses were performed where appropriate by calculating minimum detectable differences for nonsignificant differences. For minimum detectable differences, we assumed that there were 21 independent observations (\( \alpha = .05/5 \) or .05/6 depending on the comparison, \( \beta = .20 \)). All data are reported as mean (SD) unless otherwise indicated.

RESULTS

PARTICIPANTS

Confocal microscopy data were unavailable for both eyes of 1 participant at 36 months after LASIK. Four eyes of 2 participants required LASIK enhancement procedures for mild undercorrections, which were similar in the fellow eyes; the enhancements occurred soon after the 12-month examination, and data for these eyes were retained for analysis at 36 months. One eye of 1 patient experienced trauma-induced recurrent erosions between 13 and 22 months after surgery; no erosions occurred after that time, and sensitivity data for this eye were included at 36 months but confocal microscopy was not performed.

In the control group, 1 participant did not return for examination at 12 months. Also, 2 participants from that group did not return at 36 months.

FLAP VARIABLES

Measured central flap thickness at 1 month was 143 (16) \( \mu m \) with the femtosecond laser and 138 (22) \( \mu m \) with the microkeratome. The actual diameter of bladeless flaps, as reported by the femtosecond laser, was 8.9 (0.2) mm, and the intended diameter of microkeratome flaps was 9.3 (0.4) mm (the actual diameter of the microkeratome flaps was not measured); the mean difference was 0.4 (0.4) mm (\( P < .001 \)).

SUBBASEL NERVE DENSITY

Subbasal nerve density did not differ between femtosecond and microkeratome treatments at any examination before or after LASIK (Figure 1 and Table 1). For both treatments, subbasal nerve density was decreased at 1 month after LASIK (\( P < .001 \)) and remained decreased through 12 months (\( P < .001 \)). At 36 months, subbasal nerve density did not differ from the preoperative density for either treatment; minimum detectable differences were 7081 \( \mu m/mm^2 \) and 7930 \( \mu m/mm^2 \) for femtosecond laser and mechanical microkeratome, respectively (\( \alpha = .05/5, \beta = .20 \), paired analyses).

CORNEAL SENSITIVITY

Absolute mechanical thresholds did not differ between femtosecond and microkeratome treatments at any examination before or after LASIK and did not differ at any examination after LASIK compared with before LASIK within each treatment (Figure 2 and Table 2). In the concurrent controls, absolute mechanical threshold remained stable except for an increase at 36 months compared with 12 months (\( P = .01 \); Figure 2 and Table 2). As a result, we calculated mechanical threshold ratios for the LASIK eyes and found no difference between treatments at any examination and no difference between preoperative and postoperative values within treatments (Figure 3 and Table 2). Absolute mechanical thresholds also did not differ between LASIK and control eyes at any examination (\( P \geq .21 \)).

When fellow eyes of each participant were combined, the mechanical threshold ratio increased (decreased sensitivity) at 1 month (1.29 [0.85]) after LASIK compared with the preoperative value (0.89 [0.73], \( P = .05 \)), returned to the preoperative value by 3 months (\( P = .99 \)), and remained stable thereafter (42 eyes, gen-
Corneal subbasal nerve regeneration after LASIK did not differ between eyes with the flap created by the femtosecond laser and eyes with the flap created by the mechanical microkeratome. Similarly, the changes in corneal sensitivity after LASIK did not differ by treatment, and the recovery of corneal sensitivity did not correspond to subbasal nerve regeneration.

Corneal stromal nerves course anteriorly in the anterior stroma to pierce the Bowman layer and form the subbasal nerve plexus at the basal aspect of the basal epithelial cells. The nerves are devoid of myelin sheaths to aid corneal transparency and are surrounded by Schwann cells only. Stromal reinnervation after corneal wounding is facilitated by apposition of wound edges, and this might be explained by the realignment of proximal and distal Schwann cell channels. As a result, reinnervation after penetrating keratoplasty, in which Schwann cell channels do not align, is virtually absent. Central subbasal and stromal nerve fiber bundles are also virtually absent immediately after LASIK because of transection of nerves during flap creation and subsequent stromal photoablation. The nerves regrow after LASIK, although through 5 years their density remains less than it is preoperatively; regrowth might be aided by good realignment of the Schwann cell channels at the flap margin. Femtosecond lasers create planar flaps with precise and defined side-cut angles; thus, the periphery of the flap is as thick as the center and has an almost vertical edge. This geometric configuration should allow more precise realignment of femtosecond laser flaps compared with microkeratome flaps, which have tapered edges. Nevertheless, our results showed that femtosecond laser flaps did not result in faster reinnervation. Our results were not confounded by creating flaps of different thicknesses because the measured flap thickness was similar between treatments (approximately 140 µm).

Subbasal nerves were not visible in most eyes at 1 month after LASIK, and recovery of nerve density was slow, similar to that in previous studies. Although we did not show a difference in subbasal nerve density at 36 months after LASIK compared with the preoperative state, the large standard deviation of nerve density and the small sample size prevented us from detecting differences smaller than approximately 7000 µm/mm²; previously, we found that subbasal density remained lower through 5 years after LASIK. Of note, subbasal nerve density in our previous study appeared lower than that in the present study because we used confocal microscopes with differences in their optical design.

Corneal mechanical sensitivity did not differ between methods of flap creation at any examination. Lin
et al\textsuperscript{32} suggested that corneal sensitivity recovered faster after LASIK with femtosecond laser flap creation compared with microkeratome flap creation; they found a difference in central sensitivity of 3-mm filament length by Cochet-Bonnet esthesiometry at 3 months in a small nonrandomized series of eyes. In our study, corneal mechanical sensitivity was decreased at 1 month after LASIK before returning to preoperative sensitivity by 3 months. Stapleton et al\textsuperscript{26} found similar results in a cross-sectional study with the same gas esthesiometer, and Darwish et al\textsuperscript{27} found similar results by using a different noncontact esthesiometer. Gallar et al\textsuperscript{28} used the same esthesiometer as was used in our study and concluded that there was a transient hyperesthesia within days after LASIK followed by reduced mechanical and chemical sensitivity for as long as 2 years after LASIK, although their data were not statistically different from those for controls. Although our absolute mechanical threshold data suggested a trend toward hyperesthesia at 3 to 12 months after LASIK compared with controls (Figure 2), the differences were not statistically significant.

Most other studies have used Cochet-Bonnet esthesiometry to measure corneal sensitivity after LASIK.\textsuperscript{20} With this method, Chuck et al\textsuperscript{30} found that corneal sensation transiently decreased after LASIK but returned to preoperative sensitivity by 3 weeks after surgery. In contrast, most studies reported decreased sensation through at least 6 months after LASIK.\textsuperscript{21,31-34} Mian et al\textsuperscript{35} showed decreased sensation throughout the first year after LASIK. Although Cochet-Bonnet esthesiometry is a crude method of measuring corneal sensitivity and is limited by ceiling and floor effects when corneal sensitivity is normal or very abnormal, it is a quick and easy test in clinical studies and has detected a reduction in corneal sensitivity after LASIK in several studies. Gas esthesiometry has been suggested to be more sensitive than Cochet-Bonnet esthesiometry for measuring mechanical sensitivity,\textsuperscript{11,28} but our results and those of Stapleton et al\textsuperscript{26} and Darwish et al\textsuperscript{27} suggest that gas esthesiometry might in fact be less sensitive than Cochet-Bonnet esthesiometry. The discrepancy might be explained by differences in the types of receptor and the geometric area stimu-

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**Table 2. Absolute Threshold of Mechanical Sensitivity and Mechanical Threshold Ratio\textsuperscript{a}**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time After LASIK, mo</th>
<th>Preoperative</th>
<th>1 mo</th>
<th>3 mo</th>
<th>6 mo</th>
<th>12 mo</th>
<th>36 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Femtosecond laser</td>
<td></td>
<td>65 (56)</td>
<td>86 (59)</td>
<td>47 (24)</td>
<td>45 (16)</td>
<td>44 (13)</td>
<td>101 (73)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[0.97 (0.83)]</td>
<td>[1.41 (0.97)]</td>
<td>[0.77 (0.64)]</td>
<td>[0.74 (0.37)]</td>
<td>[0.60 (0.42)]</td>
<td>[1.13 (0.81)]</td>
</tr>
<tr>
<td>Mechanical microkeratome</td>
<td></td>
<td>55 (43)</td>
<td>71 (43)</td>
<td>55 (26)</td>
<td>45 (21)</td>
<td>47 (26)</td>
<td>102 (72)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>[0.82 (0.63)]</td>
<td>[1.16 (0.72)]</td>
<td>[0.91 (0.42)]</td>
<td>[0.74 (0.34)]</td>
<td>[0.87 (0.48)]</td>
<td>[1.14 (0.80)]</td>
</tr>
</tbody>
</table>

**Absolute Mechanical Threshold, mL/min**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time After LASIK, mo</th>
<th>Preoperative</th>
<th>1 mo</th>
<th>3 mo</th>
<th>6 mo</th>
<th>12 mo</th>
<th>36 mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n=40 eyes)</td>
<td></td>
<td>68 (54)</td>
<td>61 (42)</td>
<td>NA</td>
<td>NA</td>
<td>55 (32)</td>
<td>89 (68)</td>
</tr>
<tr>
<td>P value, comparing all LASIK eyes with controls</td>
<td>0.99</td>
<td>.89</td>
<td>.99</td>
<td>.24</td>
<td>.99</td>
<td>.99</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** LASIK, laser in situ keratomileusis; NA, not available.

\textsuperscript{a}Mechanical threshold ratio was defined as the ratio of mechanical threshold in LASIK corneas to that in control corneas at the concurrent examination. Control individuals were not examined at 3 or 6 months, and for these points in time, ratios were calculated by using the control data at 1 month.

\textsuperscript{b}Absolute thresholds after LASIK did not differ from thresholds before LASIK for either treatment at any examination. Postoperative threshold ratios did not differ from preoperative for either treatment at any examination; threshold ratios at 1 month compared with the preoperative examination, P=.65 (femtosecond laser) and P=.39 (microkeratome), and minimum detectable differences (\(\alpha=.05/5\), \(\beta=20, 21\) eyes, paired analyses) were 1.03 (femtosecond laser) and 0.65 (microkeratome).

\textsuperscript{c}P values comparing LASIK treatments were from paired t tests (unless stated), and P values comparing combined LASIK eyes to controls were from generalized estimating equation models; all P values were adjusted for 6 comparisons by the Bonferroni technique.

\textsuperscript{d}Wilcoxon signed rank test.

\textsuperscript{e}Thirty-eight eyes because 1 participant did not return for examination.

\textsuperscript{f}Thirty-six eyes because 1 participant at 12 months and 2 at 36 months did not return for examination.

\textsuperscript{g}P=.01 vs 12 months.

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**Figure 3. Mechanical threshold ratio before and after laser in situ keratomileusis (LASIK).** The mechanical threshold ratio is the ratio of absolute mechanical threshold in corneas undergoing LASIK to absolute mechanical threshold in control corneas and was calculated to account for the drift in absolute mechanical threshold noted in control corneas between 12 and 36 months (see Figure 2). The mechanical threshold ratio did not differ between femtosecond laser (bladeless) and mechanical microkeratome treatments at any examination before or after LASIK. The transient increase in the mechanical threshold ratio at 1 month after LASIK was not statistically significant within each treatment group (femtosecond laser, P=.65; microkeratome, P=.39) but, when all eyes were combined, the increase from the preoperative ratio was significant (P=.05), i.e., decreased sensitivity, and returned to the preoperative ratio by 3 months (P=.99) with no subsequent change.
lated by the 2 esthesiometry techniques. We also found that mechanical sensitivity significantly decreased between 12 and 36 months in the concurrent, unoperated-on control corneas; although this might be explained by interobserver variation because our observer changed between these examinations, the new observer used the same protocol as the previous one, and thus we cannot rule out a change in the gas esthesiometer. Had we not examined concurrent controls, we might have incorrectly concluded that corneal sensitivity decreased between 12 and 36 months after LASIK (Figure 2). With the gas esthesiometer, we found that the best control for an eye was its fellow eye (Table 2), and thus our conclusion of no difference in corneal sensitivity between methods of flap creation is valid.

The prolonged decrease in subbasal nerve density after LASIK was accompanied by only a transient decrease in corneal sensitivity at 1 month, and, in contrast to Lee et al., we did not find a correlation between subbasal nerve density and sensitivity at any examination. Although it is possible that the cornea is reinnervated by small-caliber nerves that are not visible with our confocal microscope, thus restoring sensation to the cornea, it is also possible that our inability to detect small changes in sensitivity after LASIK resulted in our inability to detect a relationship between nerve density and sensitivity.

Between 12 and 36 months, fellow eyes of 2 participants received LASIK enhancements, and we retained the data from these eyes at 36 months because the fellow eyes were treated for similar undercorrections, even though enhancements result in severing and ablation of corneal nerves and likely a change in corneal sensitivity. One eye of another participant experienced corneal erosions between the 12- and 36-month examinations; confocal microscopy was not performed, but the corneal sensitivity data were retained in the analysis at 36 months. We analyzed our data at 36 months after excluding the enhanced eyes and the eye with erosions (data not shown), and the results did not alter any of our conclusions.

In summary, we found that the method of flap creation did not affect the rate of corneal reinnervation or recovery of corneal sensitivity after LASIK. We were unable to find a relationship between corneal subbasal nerve density and corneal sensitivity.

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REFERENCES


