Electromyographic Differences Between Normal Upper and Lower Facial Muscles and the Influence of Onabotulinum Toxin A

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**Importance:** Empirically determined doses of onabotulinum toxin A for aesthetic treatments are as much as 5 times higher for the upper than for the lower facial muscles.

**Objective:** To use electromyography (EMG) to determine objectively whether the disparity between doses is due to intrinsic differences between the muscle groups’ responses to onabotulinum toxin A or to variable amounts of paralysis required to achieve the desired aesthetic outcomes.

**Design:** We collected EMG data before and at 2 to 4 weeks and 3 months after 8- and 2-U onabotulinum toxin A injections to the corrugator and depressor anguli oris muscles, respectively.

**Setting:** A private oculofacial plastic surgery practice.

**Participants:** Twenty-six subjects recruited from February 1 through April 1, 2009.

**Interventions:** Electromyography recordings and cosmetic onabotulinum toxin A injections.

**Main Outcome Measures:** Mean motor unit (MU) durations and maximal amplitudes at baseline and 2 to 4 weeks and 3 months after injection.

**Results:** Baseline mean MU amplitudes were similar for the corrugator and depressor anguli oris muscles. At 2 to 4 weeks after injection, 78% MU and 64% maximal amplitude reduction for the corrugator muscle were detected, but only 54% MU and 18% maximal amplitude reduction for the depressor anguli oris ($P=2.7	imes10^{-4}$ and $P=1.3\times10^{-14}$, respectively). At 3 months, function was partially recovered for both muscle groups.

**Conclusions and Relevance:** Onabotulinum toxin A causes a similar dose-dependent reduction in MU and maximal voluntary amplitudes for muscles of the upper and lower face. The dose disparity appears to result from differences in the amount of paralysis required to achieve desirable aesthetic results.

**Level of Evidence:** 2.


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The use of botulinum toxin for the treatment of facial rhytids was first described in 1992. Onabotulinum toxin A (Botox; Allergan, Inc) was approved by the US Food and Drug Administration for treatment of glabellar rhytids in 2002. According to the 2008 American Society for Aesthetic Plastic Surgery survey, onabotulinum toxin A injection is the primary nonsurgical cosmetic procedure in the United States, with more than 2.4 million treatments.

Although onabotulinum toxin A is the only treatment approved by the US Food and Drug Administration for cosmetic use on glabellar rhytids, other facial muscle groups are injected for off-label aesthetic improvements. Recommendations for doses and injection locations have been determined empirically by experts with significant injection experience and published for the upper and lower face. Acceptable and recommended doses are significantly (3-5 times) higher for the muscles of the upper face than the lower face. For the glabellar complex, doses range from 10 to 40 U, with slightly higher doses for men. In a typical 20-U glabellar treatment, each corrugator muscle is injected with 8 U and the procerus muscle with 4 U. However, to treat the similar-sized depressor anguli oris (DAO) muscles of the lower face, typically only 2 to 3 U per side is injected.

The reason for this several-fold difference in dose between the upper and lower facial muscles is poorly understood. Two
possibilities exist. Intrinsic differences in the motoneurons between the 2 anatomic locations may exist with respect to their susceptibility to onabotulinum toxin A chemodenervation. This possibility is supported by known anatomic differences in central innervation pathways between the upper and lower facial muscles; the corticopontine innervation of the upper face (frontalis and orbicularis oculi muscles) is bilateral, whereas that of the lower facial muscles is mainly contralateral. An alternative hypothesis for the dosing disparity is that, although the amount of chemodenervation is dose dependent, the optimal desired effect on each muscle group is different. The upper face may require significantly more paralysis than the lower face to achieve acceptable cosmetic results.

Although electromyography (EMG) has been used extensively for facial muscle localization before onabotulinum toxin A treatments, few studies have used EMG to ascertain the effects of treatments on targeted muscles. In this study, we examined the EMG characteristics of the corrugator and DAO muscles before and after cosmetic onabotulinum toxin A treatment to elucidate better the cause of the dose disparity between the upper and lower face.

**METHODS**

Independent review board approval was obtained (Western Institutional Review Board, Olympia, Washington; study No. 1099356), and the protocol followed the tenets of the Declaration of Helsinki. Subjects were recruited for participation from February 1 through April 1, 2009. All subjects provided full written informed consent as required by the Western Institutional Review Board before participating in the study. Subjects were excluded from participation if they were minors, had received prior onabotulinum toxin A treatments, had any known neurological disease, or had undergone surgery involving the glabella or perioral areas.

After informed consent, the skin above the corrugator and DAO muscles was prepped with alcohol. Electromyography (5200A; Cadwell Laboratories, Inc) recordings were taken from each corrugator and DAO muscle, and we analyzed the resting state, motor unit (MU), and maximal voluntary activated state. We used 28-gauge monopolar needles as the active electrode, with surface electrodes for the reference and the ground. Low- and high-pass filters were set at 320 and 10,000 Hz, respectively. Peak-to-trough amplitude and duration were recorded for the MU. The maximal voluntary amplitudes were recorded and graded on a scale of 1 to 10 by 2 independent and blinded graders (B.J.W. and B.S.S.) based on a standardized scale (Figure 1). Agreement of the readings of maximal amplitude by the 2 graders was excellent ($R^2=0.98$). We used the square of the Pearson product–moment correlation coefficient ($R^2$) to quantify correlations and the paired $t$ test to calculate $P$ values throughout the analysis unless otherwise stated.

After the initial EMG recordings, each subject was treated with 20 U of onabotulinum toxin A (Botox; Allergan, Inc) to the corrugator-procerus muscle complex (8 U to each corrugator muscle and 4 U to the procerus muscle) and 2 U to each DAO muscle (Figure 2). Doses and locations for injection were based on previously published standards. Subjects returned at the time of expected peak onabotulinum toxin A effect (2-4 weeks) and at 3 months for additional EMG recordings using the same protocol. Facial photographs were taken at all 3 encounters.

**RESULTS**

Twenty-six subjects met inclusion criteria and entered the study, including 24 women and 2 men. The mean age was 42.4 (range, 23-65; SD, 12.9) years. Two subjects did not return after their initial visit, and 2 additional subjects did not return for their 3-month visit.

**BASELINE EMG**

The mean MU amplitudes for the corrugator and DAO muscles were 119.34 and 119.64 µV, respectively ($P=.98$). Mean MU durations for the corrugator and DAO muscles were 4.58 and 4.38 milliseconds, respectively ($P=.45$). The mean maximal amplitudes were graded as 8.44 for the corrugator and 7.38 for the DAO. This difference was significant at $P=5.7 \times 10^{-6}$ (Table).

**2- TO 4-WEEK EMG**

The corrugator and DAO muscles showed a significant decrease in MU and maximal amplitudes with respect to baseline; however the change was greater for the corrugator than for the DAO muscles. The mean MU amplitudes for the corrugator and DAO muscles were 25.78 and 54.47 µV, respectively ($P=2.7 \times 10^{-3}$). These measurements represented 78% (66% when calculated using normalized data) and 55% (54% when calculated using normalized data) decreases in MU amplitudes compared with baseline for the corrugator and DAO muscles, respectively. Statistically significant differences were similarly found for the mean change in MU amplitude compared with baseline between corrugator and DAO muscles ($P=0.62$ for both). Mean MU duration was 2.60 and 3.19 milliseconds for the corrugator and DAO muscles, respectively ($P=0.01$). Mean corrugator and DAO maximal amplitudes were graded as 3.04 and 6.06, respectively ($P=1.3 \times 10^{-17}$). These grades represented 64% and 8% reductions from baseline maximal amplitudes for the corrugator and DAO muscles, respectively. Mean change in maximal amplitude relative to baseline was 5.47 and 0.91 for the corrugator and DAO muscles, respectively ($P=1.4 \times 10^{-17}$). Similarly, mean normalized maximal relative amplitude was 0.37 and 0.89 for the corrugator and DAO muscles, respectively ($P=3.4 \times 10^{-17}$) (Table).

**3-MONTH EMG**

Although the MU and maximal amplitudes were still lower for the corrugator than for the DAO muscles at 3 months, this difference was statistically significant for the maximal amplitudes only. The mean MU amplitudes for the corrugator and DAO muscles were 69.99 and 81.81 µV, respectively ($P=.20$). These measurements represented 41% and 32% decreases in MU amplitudes compared with baseline for the corrugator and DAO muscles, respectively. No significant differences were found for the mean change in MU amplitude with respect to baseline and the mean normalized
MU amplitude relative to baseline between the corrugator and DAO muscles ($P = .10$ and $P = .08$, respectively). Mean MU durations were 4.11 and 3.57 milliseconds for corrugator and DAO muscles, respectively.

Figure 1. Standard electromyography tracings used for grading (1-10) maximal voluntary corrugator and depressor anguli oris muscle amplitudes.
Mean corrugator and DAO maximal amplitudes were graded as 5.49 and 7.22, respectively ($P = 2.6 \times 10^{-6}$). Mean changes in the maximal amplitude relative to baseline were 2.97 and 0.04 for the corrugator and DAO muscles, respectively ($P = 6.7 \times 10^{-9}$). Mean normalized maximal relative amplitudes were 0.67 and 1.00 for the corrugator and DAO muscles, respectively ($P = 1.8 \times 10^{-8}$) (Table).

Trends in MU and normalized MU amplitudes are summarized in Figure 3. Maximal amplitude and normalized maximal amplitude trends are illustrated in Figure 4. For both muscle groups, the greatest reduction in MU and maximal amplitudes (raw and normalized) occurred at 2 to 4 weeks, with partial recovery at 3 months. In all cases, the amplitude reduction effect of onabotulinum toxin A on the corrugator muscles was greater than that on the DAO muscles. This difference appeared to be dose related because each corrugator muscle received 8 U of onabotulinum toxin A and each DAO muscle received 2 U.

Motor unit duration was similarly shortened most at 2 to 4 weeks after onabotulinum toxin A injection, with partial recovery at 3 months (Figure 5). We found little overall correlation between MU amplitude and MU duration ($R^2 = 0.15$). However, we found moderate correlation between MU amplitude and MU duration ($R^2 = 0.43$) and between maximal amplitude and MU duration ($R^2 = 0.42$) for the corrugator muscle at 2 to 4 weeks after onabotulinum toxin A injection but not for other variables at any of the measurement times. The fact that this correlation between amplitude and duration is seen only with the maximal onabotulinum toxin A effect and the higher dose suggests that the correlation is not truly between amplitude and duration but may be the result of simultaneous effects of onabotulinum toxin A on both variables. We found no correlation between subject age and MU amplitude, MU duration, or maximal amplitude before or after onabotulinum toxin A treatment.

When asked specifically, no patients experienced any adverse effects of the onabotulinum toxin A injections to the glabella. However, 2 patients reported transient (<1 week) difficulty in drinking through a straw related to the DAO treatment at approximately week 3 after injection.

Onabotulinum A toxin causes chemical denervation and paralysis of associated muscles by preventing the release of membrane-bound acetylcholine at the neuromuscular junction. The toxin is rapidly and irreversible

<table>
<thead>
<tr>
<th>Time From Injection</th>
<th>Muscle, Mean Value (95% CI)</th>
<th>Corrugator</th>
<th>DAO</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td>119.34 (104.46 to 134.22)</td>
<td>119.64 (99.34 to 139.95)</td>
<td>.98</td>
</tr>
<tr>
<td></td>
<td>Mean MU amplitude, µV</td>
<td>4.58 (4.18 to 4.98)</td>
<td>4.38 (4.04 to 4.71)</td>
<td>.45</td>
</tr>
<tr>
<td></td>
<td>Mean MU duration, ms</td>
<td>8.44 (8.08 to 8.79)</td>
<td>7.38 (7.14 to 7.61)</td>
<td>5.7 x 10^{-6}</td>
</tr>
<tr>
<td><strong>2-4 wk</strong></td>
<td>Mean MU amplitude, µV</td>
<td>25.78 (19.18 to 32.39)</td>
<td>54.47 (48.02 to 60.92)</td>
<td>2.7 x 10^{-8}</td>
</tr>
<tr>
<td></td>
<td>Mean change in MU amplitude from baseline, µV</td>
<td>95.03 (77.71 to 112.35)</td>
<td>62.15 (42.04 to 82.25)</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>Mean normalized MU amplitude relative to baseline</td>
<td>0.33 (0.17 to 0.49)</td>
<td>0.54 (0.46 to 0.62)</td>
<td>.02</td>
</tr>
<tr>
<td></td>
<td>Mean MU duration, ms</td>
<td>2.60 (2.23 to 2.98)</td>
<td>3.19 (2.92 to 3.46)</td>
<td>.01</td>
</tr>
<tr>
<td></td>
<td>Mean maximal amplitude grade</td>
<td>3.04 (2.57 to 3.51)</td>
<td>6.06 (5.61 to 6.51)</td>
<td>1.3 x 10^{-14}</td>
</tr>
<tr>
<td></td>
<td>Mean change in maximal amplitude from baseline</td>
<td>5.47 (4.84 to 6.10)</td>
<td>0.91 (0.42 to 1.41)</td>
<td>1.4 x 10^{-12}</td>
</tr>
<tr>
<td></td>
<td>Mean normalized maximal amplitude relative to baseline</td>
<td>0.37 (0.30 to 0.44)</td>
<td>0.89 (0.82 to 0.95)</td>
<td>3.4 x 10^{-13}</td>
</tr>
<tr>
<td><strong>3 mo</strong></td>
<td>Mean MU amplitude, µV</td>
<td>69.99 (55.61 to 84.36)</td>
<td>81.81 (71.21 to 92.40)</td>
<td>.20</td>
</tr>
<tr>
<td></td>
<td>Mean change in MU amplitude from baseline, µV</td>
<td>52.50 (28.44 to 76.55)</td>
<td>25.91 (6.59 to 45.23)</td>
<td>.10</td>
</tr>
<tr>
<td></td>
<td>Mean normalized MU amplitude relative to baseline</td>
<td>0.70 (0.53 to 0.87)</td>
<td>0.90 (0.75 to 1.06)</td>
<td>.08</td>
</tr>
<tr>
<td></td>
<td>Mean MU duration, ms</td>
<td>4.11 (3.62 to 4.59)</td>
<td>3.57 (3.24 to 3.90)</td>
<td>.08</td>
</tr>
<tr>
<td></td>
<td>Mean maximal amplitude grade</td>
<td>5.49 (4.95 to 6.04)</td>
<td>7.22 (6.84 to 7.61)</td>
<td>2.6 x 10^{-6}</td>
</tr>
<tr>
<td></td>
<td>Mean change in maximal amplitude from baseline</td>
<td>2.97 (2.27 to 3.68)</td>
<td>0.04 (0.44 to 0.52)</td>
<td>6.7 x 10^{-3}</td>
</tr>
<tr>
<td></td>
<td>Mean normalized maximal amplitude relative to baseline</td>
<td>0.67 (0.59 to 0.74)</td>
<td>1.00 (0.94 to 1.07)</td>
<td>1.8 x 10^{-1}</td>
</tr>
</tbody>
</table>

Abbreviations: DAO, depressor anguli oris; MU, motor unit.
ibly bound to the presynaptic motoneuron, internalized, and then free to act on a zinc-dependent endoprotease to inhibit acetylcholine release. Peak paralytic effect occurs by 2 to 3 weeks, and muscular function typically starts to return by 3 months. The EMG MU is defined as the anterior horn cell, its axon, and all the muscle fibers that the neuron innervates. Electromyography enables the examiner to determine the effects of a disease state (or iatrogenic manipulation) on the neuron and muscle. Although EMG has been used extensively for precise localization of muscles before onabotulinum toxin A injection, very few studies have used EMG to evaluate the effects of onabotulinum toxin A after cosmetic injection of healthy subjects. Lee and colleagues used EMG to quantify the effect of 25 U of onabotulinum toxin A to the masseter muscle to treat the cosmetically unacceptable square face. In their study, Lee et al found that the electrophysiologic effect of onabotulinum toxin A on the muscle peaked at 4 weeks (similar to our findings) and did not return to normal until 7 months after treatment. In contrast, the reduction in masseter muscle volume resulting from the treatment peaked at 3 months after treatment and persisted to 12 months. Karsai et al used EMG in a randomized double-blind study comparing 2 onabotulinum toxin A products (Botox and Dysport [Ipsel, Ltd]) for treatment of forehead wrinkles. Twelve units of Botox were compared with 36 U of Dysport injected into the frontalis muscle. The peak effect on the EMG

![Figure 3. Mean motor unit (MU) amplitude (A) and mean normalized MU amplitude (B) by time from onabotulinum toxin A injection for corrugator and depressor anguli oris (DAO) muscles. Error bars represent 95% confidence intervals.](image)

![Figure 4. Mean maximal (max) amplitude (A) and mean normalized max amplitude (B) by time from onabotulinum toxin A injection for corrugator and depressor anguli oris (DAO) muscles. Error bars represent 95% confidence intervals.](image)
for each treatment occurred 2 weeks after injection; however, Dysport had a greater effect than Botox beginning at week 10 after injection, suggesting that the ratio of Dysport to Botox might be smaller than 3:1.

In our study, EMG MU evaluations of the corrugator and DAO muscles revealed no significant difference between the muscle groups with respect to amplitude and duration at baseline. The maximal amplitude was slightly lower for the DAO at baseline; however, this finding may be due to subjects’ difficulties with maximal voluntary contracture of the DAO muscle, which is not a common facial expression. After 8 U of onabotulinum toxin A was injected into each corrugator muscle and 2 U into each DAO muscle, we found a significantly greater decrease in MU and maximal amplitudes for the corrugator than for the DAO muscle at 2 to 4 weeks. These findings suggest that the empirically determined 4-fold onabotulinum toxin A dose disparity between the upper and lower face is not due to a different response to onabotulinum toxin A from the muscles but may be secondary to differences in treatment goals between muscle groups.

We observed a 78% reduction in MU amplitude (66% reduction when calculated using normalized data) and 64% reduction in maximal amplitude at 2 to 4 weeks after injection of 8 U of onabotulinum toxin A to the corrugator muscle. For the DAO, we observed a 55% reduction in MU amplitude (54% when using normalized data) and an 18% reduction in maximal amplitude 2 to 4 weeks after injection of 2 U of onabotulinum toxin A. This finding suggests that, for the corrugator muscle, the treatment goal is almost complete paralysis, whereas for the DAO muscle, the goal is partial paralysis.

Although partial paralysis is desired in cosmetic treatment of the DAO muscle, too little paralysis is ineffective and too much can be associated with complications, such as flaccid cheek, incompetent mouth, difficulty with elocution, and asymmetric smile.\(^5,7\) These effects are usually secondary to diffusion of the toxin to nearby nontargeted muscle groups, such as the overlapping depressor labii inferioris.\(^5,7\) The amount of diffusion of the toxin into the tissues and the predictability of placing the toxin directly into the DAO muscle with its less obvious tissue plane may also be significant. These facts explain the narrow therapeutic window for the DAO muscle. In our study, 2 subjects experienced transient incompetent mouth when asked specifically, and we have observed transient asymmetric smile secondary to inadvertent depressor labii inferioris paralysis in patients not involved in this study who were treated with the same 2-U onabotulinum toxin A injection to the DAO muscle.

In contrast, we observed no complications associated with the 8-U onabotulinum toxin A dose to each corrugator muscle. This finding suggests that the glabella is a relatively more forgiving site for injection compared with the DAO muscle. Hence, higher onabotulinum toxin A doses resulting in greater paralysis can be tolerated without as much risk of complication. In trials by the US Food and Drug Administration for the cosmetic use of onabotulinum toxin A, eyelid ptosis was reported in 5.4% of glabellar treatments; however, when these data were analyzed, the bulk of the complications were attributed to a single injector with limited experience, suggesting a much lower actual expected complication rate.\(^7\)

Decreased MU duration was observed after onabotulinum toxin A treatment of the corrugator and DOA muscles at 2 to 4 weeks after injection (Figure 5). Motor unit duration is dictated by the number of muscle fibers associated with each motoneuron. Shorter durations are seen with smaller numbers of associated fibers and relate to the degree of synchronous activity. Therefore, 2 to 4 weeks after onabotulinum toxin A injection, fewer muscle fibers appear to be available for participation of the MU because of chemodenervation. This effect on duration was seen more with the 8-U treatment of the corrugator muscle than with the 2-U treatment of the DAO muscle and appears to be related to the amount of chemodenervation and loss of available muscle fibers. At 3 months, the MU duration was longer than at 2 to 4 weeks but not back to baseline levels.

Certain limitations exist in a study such as this where only empirically derived therapeutic doses of medication were examined. To prove that the dose-response curves are similar for muscles of the upper and lower face, multiple study arms with subtherapeutic and supra-therapeutic doses are needed. Higher doses of onabotulinum toxin A injected into the DAO muscle would undoubtedly result in significant study subject morbidity for the reasons we discussed. However, lower doses (eg, 6, 4, and 2 U) of onabotulinum toxin A to the corrugator muscle could be studied with EMG without significant untoward effect except for possible ineffective aesthetic results. This area for future study could support further the conclusions of this report.

In summary, the several-fold higher empirically derived dose of onabotulinum toxin A to the upper vs the lower face is likely owing to differences in therapeutic windows and treatment goals for these muscle groups rather than to intrinsic differences in onabotulinum toxin A response. Amplitude and duration of MU at baseline were...
not significantly different for the muscle groups; however, the decrease in amplitude in the higher-dosed corrugator muscles was significantly greater than that in the lower-dosed DAO muscles at 2 to 4 weeks after injection, suggesting a dose response. Electromyography may prove to be a valuable tool for obtaining objective electrophysiological data when evaluating new chemodenervation medications in the aesthetic and functional arenas.


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Author Contributions: Dr Winn had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Winn and Sires. Acquisition of data: Winn and Sires. Analysis and interpretation of data: Winn and Sires. Drafting of the manuscript: Winn. Critical revision of the manuscript for important intellectual content: Winn and Sires. Statistical analysis: Winn. Obtained funding: Winn and Sires. Administrative, technical, and material support: Sires. Study supervision: Sires.

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REFERENCES