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ORIGINAL ARTICLE

Quantitative evaluation of skin shrinkage associated with non-invasive skin tightening: a simple method for reproducible linear measurement using microtattoos

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Abstract

Non-invasive skin-tightening devices can induce thermal denaturation and skin shrinkage via externally applied radiofrequency emissions or high-frequency ultrasound. Therefore, the purpose of this study is to develop and test a method for measurement of skin reduction associated with application of such energy devices. Twenty-five healthy participants with mild to moderate skin laxity of the arms were enrolled. Pinpoint microtattoos were placed at each of the treatment sites to delineate two 6 × 12 cm rectangles per subject. A non-stretchable filament, tape and marking pen apparatus was used to measure the size of each rectangle before treatment and at follow-up visit by two blinded investigators. After randomization, one side received a single pass with a radiofrequency device (6.78 MHz), while the contralateral side received multiple passes. Participants underwent two treatment sessions to each side 2 weeks apart, and returned for follow-up 4 weeks after the second treatment. Length and area measurement were analyzed to assess precision and accuracy of measurements and to compare efficacy of treatment between pre- and post-treatment. Concordance correlation coefficients (CCC) demonstrated substantial inter-investigator reliability and precision in length measurements (CCC, 0.94 to 0.98 in pre-treatment; 0.95 to 0.98 in post-treatment). Measurements at the 6-week post-treatment follow-up demonstrated a statistically significant skin reduction in all six of the measured parameters. A simple skin measurement method requiring minimal instrumentation can quantitatively evaluate skin shrinkage associated with non-invasive skin-tightening devices.

Keywords Skin tightening · Non-invasive · Skin laxity · Microtattoo · Measurement

Introduction

Skin sagging or laxity is one of the signs of aging skin. Collagen degradation and other molecular changes may be causative [1, 2]. Correction of such laxity has traditionally been by surgical procedures, like rhytidectomy, and more recently by injection of the deflated skin envelope with prepackaged fillers. Now, so-called skin-tightening devices have become popular due to the promise of skin shrinkage without surgery [3]. Minimally invasive or truly non-

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invasive cosmetic procedures, like device-based skin tightening, may be preferred by patients and physicians for their decreased post-treatment recovery time and lower risk of significant adverse events [4].

Technologies underlying typical skin-tightening devices include radiofrequency energy, infrared light, and intense focused ultrasound. These devices deliver heat to the dermis and subcutaneous tissues, with collagen denaturation, increased fibroblast activity, and new collagen formation resulting in skin tightening over weeks to months [5]. Skin laxity can be decreased and skin contour improved on both the face and the body [6–9].

Measurement tools for assessing the effectiveness of skin-tightening devices remain either subjective and limited in their precision and reproducibility, or objective but of uncertain clinical relevance. Common measures are patient satisfaction questionnaires; self-assessment visual analogue scales of texture, firmness, and laxity; and blinded rater comparisons of standardized before and after photographs (e.g., Fitzpatrick wrinkle score, quartile grading scale, and skin laxity score) [10–13]. Specialized mechanical measurement modalities include devices for spectrophotometric intracutaneous analysis and those that use three-dimensional imaging [11, 14]. Efforts are ongoing to correlate the results of these objective measurement devices with clinically relevant changes.

Non-invasive skin tightening remains poorly accepted by regulatory authorities, largely as a consequence of the difficulty of quantifying this phenomenon. Indeed, it was not until late 2012 that US FDA first granted a skin-tightening device clearance for "lifting," in this case for the neck and submental area. Lack of measurement precision has likely also obstructed comparability of different tightening technologies, slowed the rate of technological advance, and confused physicians and patients.

The purpose of this study was to develop and test a novel method for measuring skin tightening. This method meets several criteria that have previously been elusive. Specifically, this method (1) uses an objective length scale, precise to millimeters; (2) permits blinding of investigators to avoid measurement bias that may otherwise occur as investigators may expect post-treatment lengths to be shorter; (3) relies on precise, fixed landmarks, delineated by India ink marks; (4) avoids permanent disfigurement of the area being measured by allowing removal of implanted tattoos by Q-switched laser after treatment; (5) is reproducible over time and by different investigators, given the specific protocol and the diagram showing the methodology; (6) is intuitive in terms of its validity and relevance to clinical benefit, as direct skin shrinkage is an outcome often sought by patients and physicians; and (7) is inexpensive and non-resource intensive, requiring very inexpensive measuring tools and a commonly available laser, as versus expensive imaging modalities.

Materials and methods

Methods

Study design Rater-blinded randomized treatment trial. Approved by the Northwestern University Institutional Review Board which was posted on clinicaltrials.gov (NCT00894244) before the start of data collection.

Subject selection Eligible subjects were aged 36–70, all female, Fitzpatrick skin types I–III, with mild to moderate skin laxity of the arms, and presenting to the dermatology clinical service at Northwestern University. Exclusion factors included systemic illness, abnormal wound healing, history of hypertrophic scars, local skin disease affecting the arms, or skin too dark to visualize microtattoos placed for orientation. A written informed consent was obtained from all subjects (Fig. 1).

Materials and equipment The skin-tightening device used was a monopolar radiofrequency device (Thermacool, Solta Medical, Hayward, CA) with frequency of 6.78 MHz that generated heat in tissue by through an electric field that rapidly changes polarity. The treatment tip was a ThermaTip DC (deep contouring) 3-cm^2 attachment (300–600 shots per tip). Specialized black tattoo ink (Infinitink, Freedom-2™, Cherry Hill, NJ) that is microencapsulated for rapid removal by 1064-nm Q-switched Nd:Yag laser (Medlite, Cynosure, Inc., Westford, MA) was used to place microtattoos for orientation and to assist in measurement. As per DiBernardo, prior to treatment, the treatment area was divided into 12 × 6-cm rectangles outlined by a surgical marker pen. The corners of each rectangle were tattooed with India ink, by dipping in a sterile contained and delivering to the skin surface by dermal puncture with an 18-gauge needle [15].

Randomization protocol For each study participant, a random number table was used to produce two consecutive numbers, each either a 1 or a 2 (i.e., possible combinations 1 and 2, 2 and 1, 1 and 1, and 2 and 2). The first number denoted whether treatment would occur on the medial (1) or posterior (2) arms. The second number indicated whether the right arm would receive one treatment pass of the radiofrequency device (1) or two to five passes, as tolerated (2). If the right arm received one pass, the left would receive 2–5; if the right received 2–5, the left would receive one. Allocation sequence generation (N.A.K.) was done by a different investigator than participant enrollment and assignment (S.N.).

Once the pairs of numbers were created, each pair was inscribed on a notecard, and each of the notecards was sealed in an opaque envelope. Envelopes were shuffled and then numbered consecutively, and were used as such for consecutive enrolled subjects.

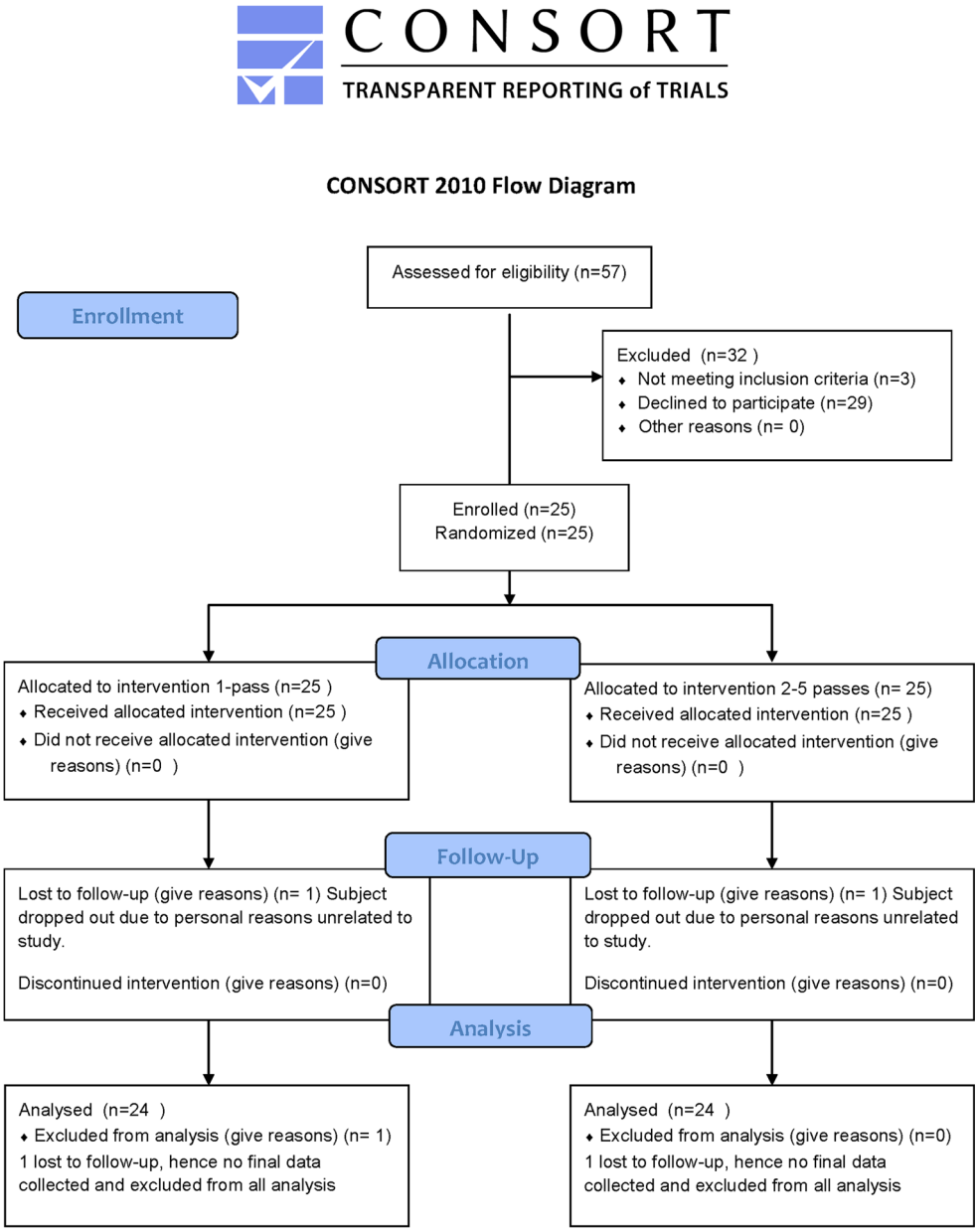

Fig. 1 The CONSORT 2010 flow diagram of participants

Participants served as their own controls to minimize errors and inconsistencies related to differences in skin type, elasticity, skin thickness, and age. Right/left arm randomization was used to further mitigate the risk of systematic outcome differences based on sun-exposed or dominant arms being preferentially allocated to a given study group. These randomization and control practices are consistent with prior dermatologic studies [16–18].

Masking Participants and investigators administering the treatments (M.P.) were not blinded as they could feel and see the difference between one and multiple passes, and could identify the anatomic location of the treatment. Those assessing the outcomes (S.N., N.A.K.) were blinded as to assignment.

Microtattoo placement and skin measurement protocol Each subject received two sets of four microtattoos to demarcate the two treatment sites on their upper inner left and right arms. At each arm, two of these small (1–2 mm) circular tattoos were placed approximately 6 cm apart along a short axis and two were placed 12 cm apart on a long axis. Subjects were tattooed with their arms completely relaxed, and the elbow was fully extended. The lower edge was 10 cm away from the olecranon process of the ulna.

All study personnel applying microtattoos received hands-on training from the chief technician responsible for placing radiation tattoos at Radiation Oncology Center, Northwestern Memorial Hospital. The specific details of the process were as follows: (1) don a pair of sterile gloves, (2) uncap tattoo ink

and use the dropper to place one drop of ink on the point to be tattooed; do not touch dropper to patient's skin, (3) uncap 18-gauge needle, (4) with beveled edge up, press needle to puncture skin through the ink into the dermis; turn the needle one half turn and withdraw the needle, (5) clean tattooed area with alcohol pad, and (6) dry tattooed area with sterile gauze.

Before measurement, the arm to be measured was extended horizontally. For each of the rectangles defined by a set of four microtattoos on the respective vertices, each linear segment between each pair of dots was measured using an unmarked non-stretchable filament that was pulled taut. The filament was taped with a clear adhesive to one microtattoo, and held, firmly but without pulling, to the other microtattoo. Throughout its course between the tattoos, the filament was touching but not pressed into the skin. Skin folds were not manually stretched out, with any stretching solely attributable to gravity along the perpendicular axis. Since the tattoo dots had a measurable width (1 mm), for consistency, measurements were taken from the outside edge of the dots closest to the outside edge of the corresponding second dot defining the line segment to be measured. Using an ultrafine ink pen (0.1 mm line), marks were made on the filament to denote this distance, with again the outside edges of the two marks defining the length. Collectively, each measurement cycle required six measurements, including the two short axes of the perimeter (width 1, width 2), the two long axes of the perimeter (length 1, length 2), and the two diagonals through the rectangle (diagonal 1, diagonal 2) (Fig. 2). Each blinded rater obtained one measurement twice for each of the six measurements both before the first treatment and 6 weeks later. Numerical measurements were obtained from the inked filaments by placing each such filament along a rigid measuring stick.

Treatment protocol Once subjects were enrolled, randomized, tattooed, and measured, the marked anatomic sites on the contralateral arms, respectively, were treated with monopolar radiofrequency. For each patient, different treatment parameters were used for each arm: the side randomized to receive one pass was treated at level 354 with 50% overlap between adjacent spots; the other side was treated with 2–5 passes, at 353, with 20% overlap. Subjects received two treatments to each treated site, with the second treatment 2 weeks after the first. Thereafter, subjects returned 4 weeks after the second treatment for repeat measurement and laser tattoo removal.

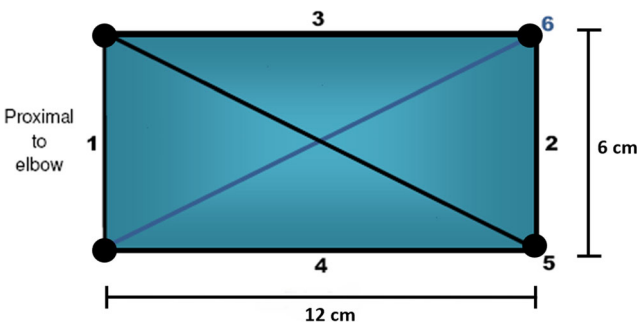

Fig. 2 A schematic diagram showing microtattoo locations (round black dots) and the associated length measurements. Measurement 1 is the width of the rectangle nearest the proximal elbow, 2 is the other width, 3 is the length at the superior border of the rectangle, 4 is the length at the inferior border, 5 is the diagonal from superior to inferior, and 6 is the diagonal inferior to superior. Measurement of the rectangle before treatment was 6 cm × 12 cm

Outcome measurement The primary outcome was the reproducibility of results obtained with the length measurements. Secondary outcomes were (1) the change in length measurements before and after treatment, and (2) differences in length measurements associated with the different treatment arms.

Sample size As this was a novel measurement algorithm, there were no pre-existing data on the precision and accuracy of the measurement technique. Therefore, we used sample size for treatment effect instead of measurement. The sample size of 25 subjects and two measures per subject would have had 80% power to detect a mean change due to treatment of 0.4 standard deviations assuming a two-tailed test and a type I error rate of 5%.

Statistical analysis Lin's concordance correlation coefficient (CCC) was calculated to assess the inter-rater reliability of each measured side [19]. A CCC value of 0 indicates complete lack of test-retest reliability and a CCC of 1 indicates perfect test-retest reliability. The paired *t* test was used to compare the pre- and post-treatment means of the six lengths. *t* tests were also used to determine whether one or 2–5 passes were more effective in reducing lengths. A repeated measure analysis of variance (ANOVA) was used to determine if the number of passes or location made a difference, with location as the between person factor, and number of passes as the within person factor. p values < 0.05 were considered statistically significant. All statistical analyses were performed with the SAS version 9.4, (SAS Institute Inc., Cary, NC).

Results

Twenty-five female subjects were enrolled in the study, and one dropped out due to personal reasons (Fig. 1). Mean subject age was 57 years (range 36–70), height was 64 in. (range 60–68), weight was 154 pounds (range 125–235), and BMI was 26 (range 21.3–35.7). No statistically significant difference in weight between pre- and post-treatment was detected. Demographic characteristics were similar for the treatment subgroups (Table 1). Two subjects declined the second treatment but did complete all other study procedures. Data was analyzed by intention to treat for the 24 subjects receiving at least one treatment.

Table 1 Demographics of study participants

Age (years)	Mean	57
	Range	36–70
Sex (no. of subjects)	Female	25
Height (inches)	Mean	64
	Range	60–68
Weight (pounds)	Mean	154
	Range	125–235
BMI	Mean	26
	Range	21.3–35.7
Fitzpatrick skin type (no. of subjects)	I	6
	II	5
	III	14

Mean measurement differences and standard deviations between investigators at pre- and post-treatment are presented in Table 2. To assess inter-rater reliability of each measurement, CCC are presented in Table 2. For the pre-treatment assessments, the coefficients ranged from 0.94 to 0.98 for all length parameters. For the post-treatment assessments, the coefficients ranged from 0.95 to 0.98. Measurements at the 6-week follow-up demonstrated a statistically significant reduction in all six of the measured parameters regardless of the number of passes (Table 3). Table 4 shows each mean length reduction by number of passes. There was no statistically significant difference in post-treatment parameter length associated with either anatomic location (i.e., either right/left, or medial/posterior), or number of passes (single/multiple passes).

Discussion

This study demonstrates that reduction in skin width and length (i.e., skin tightening of skin shrinkage) can be reliably measured by a simple, objective, reproducible, and low-cost procedure. The primary outcome was the reproducibility of the length and area measurement technique. The CCC between repeated measures ranged from 0.94 to 0.98, indicating a high level of inter-rater reliability between measurements. Mean measurements did not differ by more than 1.6 mm between raters.

Secondarily, the study also indicated that the skin-tightening device used was effective. Finding a treatment-related difference was not the primary objective of this study nor of particular interest in itself, but rather it was confirmation that the proposed measurement technique would be of utility in detecting such change. Interestingly, while both single pass and multiple pass treatments induced some skin shrinkage, there was no significant difference between single and multiple pass interventions, presumably because multiple passes did not increase treatment effectiveness.

The microtattoo procedure employed to create fixed landmarks was found to be helpful in reducing inter-rater and intra-rater measurement variation. While the microtattoo pigment used for this study was specially developed for easy removal by Q-switched laser devices, ultraviolet tattoo inks are now available that can be illuminated with a Wood's lamp and may not even require removal [20].

To minimize bias in measurements, the two investigators applying filaments to participants' microtattoos were blinded. Moreover, a different person translated the inked filament to a numerical measurement. This made it difficult, if not

Table 2 Concordance correlation coefficients (CCC) of baseline and final measurements of skin length

Measurement parameter[a]	Time	Investigator 1 measurement in mm (mean ± SD)	Investigator 2 measurement in mm (mean ± SD)	CCC
1	Baseline	60.7 ± 4.5	62.3 ± 2.9	0.94
	Final	59.5 ± 4.0	60.0 ± 4.0	0.97
2	Baseline	62.2 ± 4.6	63.3 ± 4.3	0.95
	Final	60.6 ± 4.4	61.0 ± 4.2	0.96
3	Baseline	120.2 ± 5.6	121.4 ± 4.7	0.98
	Final	117.7 ± 9.9	118.0 ± 10.1	0.97
4	Baseline	121.6 ± 8.7	122.3 ± 5.2	0.97
	Final	120.4 ± 8.0	121.4 ± 7.9	0.98
5	Baseline	135.6 ± 6.1	136.4 ± 7.4	0.96
	Final	133.9 ± 7.3	134.8 ± 7.4	0.97
6	Baseline	136.0 ± 6.5	136.7 ± 5.4	0.95
	Final	134.0 ± 5.4	134.8 ± 5.5	0.98

[a] Measurement parameters 1–6 represent the length measurements of the schematic diagram from Fig. 2. Measurement 1 is the width of the rectangle nearest the proximal elbow, 2 is the other width, 3 is the length at the superior border of the rectangle, 4 is the length at the inferior border, 5 is the diagonal from superior to inferior, and 6 is the diagonal inferior to superior

Table 3 Reduction in skin lengths at 6 weeks

Measurement parameter[a]	Mean reduction (mm)	95% CI (mm)	*p* value
1	1.0208	0.3855–1.6561	0.0022
2	1.5521	0.9868–2.1174	< 0.0001
3	2.6458	0.2621–5.0296	0.0304
4	0.9896	0.3030–1.6761	0.0057
5	1.4479	0.7277–2.1681	0.0002
6	1.8333	1.0190–2.6477	< 0.0001

[a] Measurement parameters 1–6 represent the length measurements of the schematic diagram from Fig. 2. Measurement 1 is the width of the rectangle nearest the proximal elbow, 2 is the other width, 3 is the length at the superior border of the rectangle, 4 is the length at the inferior border, 5 is the diagonal from superior to inferior, and 6 is the diagonal inferior to superior

impossible, for an investigator applying a filament to a participant's skin to consciously or unconsciously be biased by numerical measurement values obtained in other participants, as this investigator neither knew the numerical value of the initial measurement, nor had continued access to the portion of filament used to obtain that measurement.

This study has several limitations. First, this sample used was relatively homogenous in age, exclusively female, and restricted to Fitzpatrick skin types I–III. Second, only one anatomical region (the upper inner arm) was treated in this study. However, the population and anatomic site were deliberately selected to approximate real treatment conditions for skin tightening off the face. While more routine clinical skin-tightening treatments are probably performed on the neck and face, these areas are too cosmetically sensitive for the application of even temporary measurement tattoos; additionally, the lack of a broad, uniformly flat expanse of skin on the face complicates measurement and is not ideal for validating a measurement system. The use of a homogeneous sample also improved internal validity. Notably, only one non-invasive skin-tightening modality, represented by a monopolar RF device, was used in this study. But this is of lesser interest since the primary objective was to validate measurement protocols, and the general mechanism of skin reduction is similar across commercially available devices [21–23]. Another limitation is potential underlying differences in participant's skin elasticity that may have been associated with differences in age or other factors. Length measurements may have been affected by temperature gradients, as warmer skin may be measured as marginally different than cooler skin.

While this study shows a simple, reproducible method for measuring skin tightening without surgery, it is not a panacea for all measurement-associated problems. For instance, in long-term studies, patients could gain or lose weight, with this changing distance between tattoos for reasons unrelated to treatment effects [24].

In conclusion, this study demonstrates a simple skin surface measurement system that has research and clinical applications. Such a system could be adapted to measure skin surface reduction at other body sites, as well as head and neck sites. The measurement technology is not device-specific, and could theoretically be used to obtain skin lengths before and after the application of any energy device, injection, or surgical procedure designed to reduce skin surface. Additional benefits of this measurement system are that it is inexpensive, requires minimal training, and is quick and easy to apply and erase. Further studies are required to determine whether other investigators can or cannot replicate our results using

Table 4 Reduction in skin length by number of passes

Measurement parameter[a]	Passes	Mean reduction (mm)	95% CI (mm)	*p* value
1	Single	0.875	− 0.2918–2.0418	0.1345
	Multiple	1.167	0.5545–1.7788	0.0006
2	Single	1.250	0.4430–2.0570	0.0039
	Multiple	1.854	1.020–2.6882	0.0001
3	Single	1.958	1.298–2.618	< 0.0001
	Multiple	1.125	0.6498–1.6002	< 0.0001
4	Single	1.354	0.3227–2.3856	0.0123
	Multiple	0.625	− 0.3360–1.5860	0.1916
5	Single	1.479	0.5157–2.4426	0.0042
	Multiple	1.417	0.2709–2.5624	0.0176
6	Single	2.229	1.0846–3.3738	0.0005
	Multiple	1.438	0.2138–2.6612	0.0233

[a] Measurement parameters 1–6 represent the length measurements of the schematic diagram from Fig. 2. Measurement 1 is the width of the rectangle nearest the proximal elbow, 2 is the other width, 3 is the length at the superior border of the rectangle, 4 is the length at the inferior border, 5 is the diagonal from superior to inferior, and 6 is the diagonal inferior to superior

this technology, and to decide what extent this measurement should be used in combination with other measurements, like imaging tests and patient reported outcomes.

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Compliance with ethical standards

Conflict of interest Murad Alam has received research grants from Optmed in the past that are not related to this manuscript. Murad Alam is a consultant for Amway, which is not related to this manuscript. All other authors declare that they have no conflict of interest.

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