Effect of laughter exercise versus 0.1% sodium hyaluronic acid on ocular surface discomfort in dry eye disease: non-inferiority randomised controlled trial

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ABSTRACT

OBJECTIVE

To assess efficacy and safety of laughter exercise in patients with symptomatic dry eye disease.

DESIGN

Non-inferiority randomised controlled trial.

SETTING

Recruitment was from clinics and community and the trial took place at Zhongshan Ophthalmic Center, Sun Yat-sen University, the largest ophthalmic centre in China, between 18 June 2020 to 8 January 2021.

PARTICIPANTS

People with symptomatic dry eye disease aged 18-45 years with ocular surface disease index scores ranging from 18 to 80 and tear film break-up time of eight seconds or less.

INTERVENTIONS

Participants were randomised 1:1 to receive laughter exercise or artificial tears (0.1% sodium hyaluronic acid eyedrop, control group) four times daily for eight weeks. The laughter exercise group viewed an instructional video and participants were requested to vocalise the phrases "Hee hee hee, hah hah hah, cheese cheese cheese, cheek cheek cheek, hah hah hah hah hah hah" 30 times per five minute session. Investigators assessing study outcomes were masked to group assignment but participants were unmasked for practical reasons.

MAIN OUTCOME MEASURES

The primary outcome was the mean change in the ocular surface disease index (0-100, higher scores indicating worse ocular surface discomfort) from baseline to eight weeks in the per protocol population. The non-inferiority margin was 6 points of this index score. Main secondary outcomes included the proportion of patients with a decrease from baseline in ocular surface disease index score of at least 10 points and changes in dry eye disease signs, for example, non-invasive tear break up time at eight weeks.

RESULTS

299 participants (mean age 28.9 years; 74% female) were randomly assigned to receive laughter exercise (n=149) or 0.1% sodium hyaluronic acid (n=150). 283 (95%) completed the trial. The mean change in ocular surface disease index score at eight weeks was −10.5 points (95% confidence interval (CI) −13.1 to −7.82) in the laughter exercise group and −8.83 (−11.7 to −6.02) in the control group. The upper boundary of the CI for difference in change between groups was lower than the non-inferiority margin (mean difference −1.45 points (95% CI −5.08 to 2.19); P=0.43), supporting non-inferiority. Among secondary outcomes, the laughter exercise was better in improving non-invasive tear break up time (mean difference 2.30 seconds (95% CI 1.30 to 3.30), P<0.001); other secondary outcomes showed no significant difference. No adverse events were noted in either study group.

CONCLUSIONS

The laughter exercise was non-inferior to 0.1% sodium hyaluronic acid in relieving subjective symptoms in patients with dry eye disease with limited corneal staining over eight weeks intervention.

TRIAL REGISTRATION

ClinicalTrials.gov NCT04421300.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Dry eye disease is a chronic condition worldwide linked with psychological stress and poses an economic burden of long term use of artificial tears

0.1% sodium hyaluronic acid, a widely used artificial tear, has a proven therapeutic effect for ocular discomfort, and stabilising tear film in patients with dry eye disease

Laughter therapy is recognised as a beneficial complementary and adjunctive treatment for various chronic conditions, including mental health disorders, cancer, and diabetes

WHAT THIS STUDY ADDS

Laughter exercise was non-inferior to artificial tears (0.1% sodium hyaluronic acid) in improving dry eye disease symptoms and clinical signs

Laughter exercise is a safe, environmentally friendly, and low cost intervention for patients with symptomatic dry eye disease and limited corneal staining

Introduction

Dry eye disease is a widespread, complex ocular surface disease characterised by chronic subjective ocular discomfort, tear film instability, and visual disturbance.[1-3] With the population ageing, video display terminal use increasing, and air pollution, the global prevalence of dry eye disease has risen sharply, affecting approximately 360 million individuals.[4] Use of artificial tears is the mainstay treatment for dry eye disease. Although not fatal, dry eye disease substantially reduces life quality and is a substantial economic burden, particularly among long term users of artificial tears.[5] The global artificial tears market had a revenue share of USD 2.74 billion in 2022 (£2.12 billion, €2.53 billion), expected to advance to USD 4.40 billion by 2031.[6] Additionally, the societal costs such as lost work productivity and the psychological

and physical impacts of dry eye disease, are estimated at $55 billion in the US.[7]

Studies across multiple geographies and populations have reported the correlation between dry eye disease and mental health, particularly in negative emotions such as depression and anxiety.[8 9 10 11 12] In people with depression or anxiety, the link is stronger for dry eye disease symptoms as compared with patient signs.[8 10 13-15] These results suggest that the connection between dry eye disease symptoms and depression or anxiety does not appear to be driven by dry eye disease signs.[11]

In recognition of the importance of positive psychology, the World Health Organization considers happiness as a crucial component of overall well being.[16] Individuals with higher levels of subjective happiness report fewer symptoms of dry eye disease,[17 18] however, the impact of positive emotions on dry eye disease remains uncertain.

In accordance with the well known saying "laughter is the best medicine", laughter therapy was first reported by Cousins in 1979, primarily involving mimicking mouth shapes while enunciating "hahahoho."[19] Research findings suggested laughter might be therapeutic to reduce disease symptoms.[20] At present, laughter therapy comprises various interventions designed to provoke laughter, smiling, and pleasant feelings such as laughter exercises, clowns, comedy movies, games, and puzzles.[21] Abundant evidence suggests that laughter therapy alleviates depression, anxiety, stress, and chronic pain, while strengthening immune function[22-24]; and therefore, it has been recognised as a complementary and adjunctive approach for chronic conditions like mental health disorders, cancer, diabetes, among others.[25-30] Whether laughter therapy has a beneficial effect on dry eye disease, a chronic condition closely related to mental health and lifestyle,[31] remains unknown. In our pilot studies before the randomised controlled trial, we observed that laughter could immediately improve tear film stability and lipid layer thickness (protocol, pages 12-13 in supplementary file, data unpublished). Additionally, in a small scale study, we found that laughter exercise could alleviate symptoms of dry eye disease (protocol, pages 15-16 in supplementary file, data unpublished).

In this study, we conducted a randomised trial to compare the effectiveness of laughter exercise versus artificial tears in treating symptomatic dry eye disease. The hypothesis was that non-inferiority would be established by observing a between-group difference in the ocular surface disease index score of ≤6 points in eight weeks between the laughter exercise group and the controls, who were using the mainstay treatment of artificial tears. We chose a non-inferiority trial design because laughter exercise is a safe, environmentally friendly, and low cost intervention.

Methods

Study design

In this two arm, non-inferiority randomised controlled trial, the setting was at a single tertiary referral centre, Zhongshan Ophthalmic Center, Sun Yat-sen University, which is the largest ophthalmic centre in southern China. All procedures adhered to the protocol approved by the ethics committee of Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China (2020KYPJ010). Written informed consent was obtained from all participants. The trial protocol and statistical analysis plan are available in the supplementary files.

Participants

Participants with symptomatic dry eye disease were recruited from cornea and ocular surface disease clinics and locations surrounding the hospital using community advertisements, flyers, and via social media. All participants passed a screening examination 14 days before inclusion. Screening included a medical history and subjective assessment of dry eye disease symptoms using the ocular surface disease index. The international dry eye workshop in 2007 and 2017, emphasised that dry eye disease is a symptomatic disease, and symptom questionnaires are among the most reliable diagnostic tests available.[32 33] The ocular surface disease index is a 12-item index measuring ocular discomfort, with scores ranging from 0 to 100, with 0 indicating the least discomfort and 100 the greatest.

Participants were required to fulfil the diagnostic criteria of the global consensus tear film and ocular surface association dry eye workshop II for dry eye disease.[32] Participants were also required to have the ocular surface disease index score of 18 to 80 at both the screening and the eligibility confirmation visits approximately two weeks later, and a fluorescein tear break-up time (time from a blink to the appearance of the first gap in the tear film, with shorter times indicating worse tear film stability) of eight seconds or less at eligibility confirmation visit. Exclusion criteria included a score from the National Eye Institute's corneal fluorescein staining examination of more than 5 (range 0-15, with higher scores indicating more severe corneal epithelial defects); use of any dry eye disease treatment in the 14 days before enrolment; contact lens usage within 14 days before screening visit; inability to guarantee no contact lens wear for three months after trial participation; any eye surgery or history of ocular trauma within the past 12 months; any history of eye infection or allergy within the past three months; severe ocular surface scarring or conditions that could compromise the integrity of the ocular surface, such as Steven-Johnson syndrome; any previous diagnosis of glaucoma or treatment with glaucoma medications; and the presence of neurological, psychiatric or, sleep disorders. We did not enrol anyone with cognitive or comprehension deficits who could not co-operate with the eye examination, questionnaire completion, or use

of the face recognition application study application laughing face, or who had major life changes, such as planning to move to another city within the next three months, or were fearful of the bright light from eye examination equipment for this study. A complete list of the inclusion and exclusion criteria is provided in the protocol (pages 20-22).

All participants were shown the protocol and signed patient consent forms.

Randomisation and masking

Stratified block randomisation was applied with a block size of four. Participants were stratified based on their baseline ocular surface disease index scores (mild ≤18 to <23, moderate ≤23 to <33 and severe ≤33 to <80). Within each stratum, patients were randomly assigned in a 1:1 ratio to receive either laughter exercise or 0.1% sodium hyaluronic acid for eight weeks. The random sequence was generated by an independent statistician who was not involved in project implementation, using an online generator. The randomisation assignment was kept undisclosed to the trained investigators responsible for screening and outcome assessment. Participants were unmasked to group assignment but were masked to trial hypotheses.

Intervention

Drawing from previously established methods of laughter therapy, we adapted and developed the laughter exercise (supplementary video) to optimally engage the ocular muscles of participants. Participants were instructed to perform the laughter exercise four times daily for eight weeks. At the baseline visit, an instructional video showed the execution of the exercise. Specifically, laughter exercise required participants to vocalise and repeat the phrases as "Hee hee hee, hah hah hah, cheese cheese cheese, cheek cheek cheek, hah hah hah hah hah hah" 30 rounds each time, lasting for at least five minutes (video 1, video 2). To standardise the laughter exercise and enhance facial movements throughout the session, participants used a face recognition application on their mobile devices that had been designed by authors, named laughing face, at the start of each session (supplementary figure 1). The app was developed in collaboration with South China University of Technology and Xinhuixing Information Technology, Inc (Guangzhou, China). Following the eight week intervention, participants ceased the exercise and underwent follow-up at the week 10 and 12.

Participants in the control group applied artificial tears, 0.1% sodium hyaluronic acid eyedrops, to both eyes four times daily for eight weeks, tracking their usage frequency via the laughing face app. After the eight week treatment, they were instructed to discontinue the eye drops and returned for follow-up appointments at weeks 10 and 12.

The participants were followed up on weekends for their convenience. Participants in both groups were encouraged to continue their routine activities during the 12 week study period. Adherence was supported through an oral and written commitment from all participants at the baseline evaluation. For daily record keeping, participants in both study groups used the laughing face app, for which they were provided with individual access codes. The app provided daily text reminders to complete the study interventions. Those in the laughter exercise group were requested to practice with the app before each laughter session and upload their exercise videos at least once a day. All participants used the app to log their exercise sessions or eye drops use. If a participant missed an exercise session or eye drop application, they were prompted by the app to make up for the missed session. Throughout the eight week period, we tracked the number of missed exercise sessions and eye drops applications as a measure of compliance.

Outcomes

The primary outcome was the mean change in the ocular surface disease index score from baseline to eight weeks. Prespecified secondary outcomes included: the proportion of patients with a decrease from baseline in the ocular surface disease index score of 10 points or more (ie, the minimal clinically meaningful cut-off[34]), changes in signs of dry eye disease as assessed by non-invasive tear break up time, corneal fluorescein staining score, tear meniscus height, changes in the scores on the physical and mental health subscales of the 36-item short form health survey[35] (scores range from 0 to 100, with higher scores indicating better health-related quality of life), changes in the scores of self-rating anxiety scale[36] and self-rating depression scale[37] (scores range from 0 to 100, with higher indicating severe self-report anxiety or depression), changes in the scores of Pittsburgh sleep quality index[38] (scores range from 0 to 21, with higher scores indicating worse sleep quality), changes in the scores of subjective happiness scale[39] (scores range from 1 to 7, with higher scores indicating a higher level of happiness). Because of the inconsistent correlation between reported symptoms and clinical signs, we chose non-invasive tear break up time as the main secondary outcome. The details and examination procedures of exploratory outcomes including function and structure of meibomian gland were described in the trial protocol (supplementary file).

Participants were scheduled to return on day seven for assessments, after that at two weeks, four weeks, six weeks, and eight weeks (the last day of treatment and the time of assessment for the main study outcome), and again at 10 and 12 weeks. We monitored adverse events throughout the treatment period using a standard adverse event case report form at each visit.

Statistical analysis

Sample size estimation

The trial was designed to enrol 296 participants (n=148 in each group), reaching 90% statistical power to detect non-inferiority at a one-sided α=0.025 based on the primary outcome using a one sided, two sample

t-test. We selected a non-inferiority margin of 6 points, which is 50% of the minimum meaningful clinical difference on the ocular surface disease index,[32] with the common standard deviation of 15. The true difference between means was assumed to be 0.00, and loss to follow up less than 10%. Non-inferiority would be established by a one sided test of the 97.5% confidence interval (CI) of the difference in trial arms that was less than the non-inferiority margin. This margin would be equivalent to testing whether a two sided 95% CI around the treatment difference falls within the non-inferiority margin. The sample size was calculated using PASS 16.0.

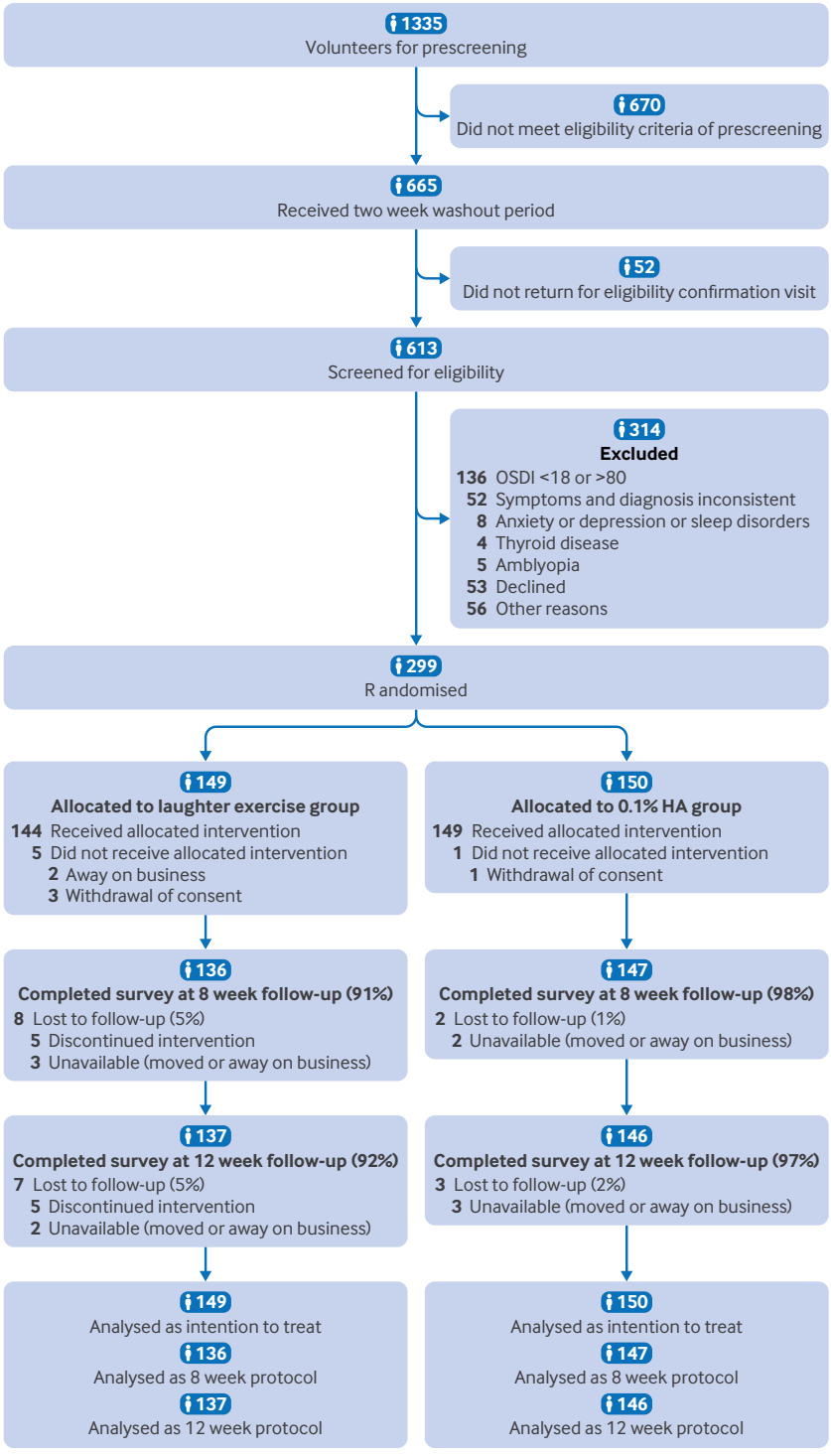

Fig 1 | Flow of participants through trial. HA=hyaluronic acid; OSDI=ocular surface disease index

Data analyses

We developed a statistical analysis plan before final analysis (supplementary file). No interim analysis was undertaken. The trial was registered at ClinicalTrials.gov (NCT04421300) on 5 June 2020. The first participant was enrolled on 3 July 2020. The participants' baseline characteristics were described as mean (standard deviation) or median (interquartile range) for continuous variables, and frequency (percentage) for categorical variables. The normality of continuous data was checked using the Shapiro-Wilk test and histograms.

Both per protocol and the intention-to-treat analyses were used in calculating the adjusted difference and 95% CI for the primary outcome, and intention to treat was used in calculating unadjusted difference and 95% CI for secondary outcomes. In intention-to-treat analysis, all participants undergoing randomisation were included, and all missing data were imputed using multiple imputations, creating 20 copies of the data. Results were obtained by averaging these 20 datasets using Rubin's rules.[40]

Line and box plots were drawn to show the outcome's change across time. Generalised estimated equation model with adjustment for intra-eye correlation was applied for the comparisons of clinical outcomes, which were measured in both right and left eyes. The difference between groups was tested using the two sample t-test for primary and psychological outcomes, and generalised estimated equation model for all clinical outcomes. Pre-post changes were tested with the paired t-test for primary and psychological outcomes and generalised estimated equation models for all clinical outcomes.

Data were cleaned using Stata16.0 and all statistical analyses were performed using SAS 9.4. A two sided P value less than 0.05 was considered statistically significant. For secondary outcomes, P for between group difference was adjusted by the Benjamini-Hochberg method with a false discovery rate of 0.05 for multiple comparisons. P values for exploratory outcomes were not calculated.

Patients and public involvement

Although patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research, they did participate in the preliminary study. Participants provided suggestions for implementing our laughter exercise, including the number of repetitions per section, the frequency of daily interventions, and how to improve communication between the research team and patients. Moreover, the results were communicated to patients who expressed an interest after the completion of the study.

Table 1 | Baseline characteristics of study participants

	Laughter exercise group (n=149)	0.1% hyaluronic acid group (n=150)
Age (years), mean (SD)	28.9 (6.33)	28.9 (6.29)
Female sex, No. (%)	109 (73)	112 (75)
Male sex, No. (%)	40 (27)	38 (25)
Education of >12 years, No. (%)	139 (93)	144 (96)
BCVA ≥0.8, No. (%)	148 (99)	148 (99)
SER for right eye (diopter), median (IQR)	−3.38 (−5.50 to −1.50)	−3.25 (−5.00 to −1.00)
OSDI grade at baseline, No. (%):		
≥18 to <23	27 (18)	27 (18)
≥23 to <33	47 (32)	48 (32)
≥33 to <80	75 (50)	75 (50)

BCVA=best corrected visual acuity; IQR=interquartile range; OSDI=Ocular surface disease index; SD=standard deviation; SER=spherical equivalent refraction.

Results

Between 18 June 2020 and 8 January 2021, 1335 volunteers were screened for eligibility (fig 1). Of these, 665 (50%) volunteers met none of the exclusion criteria and were scheduled to attend an eligibility confirmation visit two weeks later. Of the 613 (92%) people presenting for the examination, 299 (49%) were eligible for inclusion in the trial and were randomly assigned (1:1) to the laughter exercise group or the 0.1% sodium hyaluronic acid (control) group. The intention-to-treat population, therefore, included 149 participants in the intervention group and 150 participants in the control group. Table 1 shows the baseline data for the 299 participants who were randomly allocated a group. Participants had a mean age of 28.9 (6.30) years, 74% (221/299) were women, 95% (283/299) have more than 12 years of education. The high proportion of female participants is consistent with the general population in that women are more likely to have dry eye disease than men.[5 41 42] Baseline characteristics were well balanced between the two groups. In the laughter exercise group, 92% (137/149) of participants completed the scheduled follow-up visits at 12 weeks, and that in the control group was 98% (146/150). The median of the cumulative compliance during the eight week treatment was 85% (interquartile range 65-96%) for the laughter exercise group and 81% (68-90%) for the control group.

The primary outcome of mean change in the score of the ocular surface disease index from baseline to eight weeks was −10.5 points (95% CI −13.1 to −7.82) in the laughter exercise group and −8.83 (−11.7 to −6.02) in the control group. The decrease in the ocular surface disease index scores was statistically significant in both groups at eight weeks compared with baseline (both P<0.001). The upper boundary of the confidence interval for the mean between group difference in change between laughter exercise and control was lower than the non-inferiority margin of 6 points (−1.45 points (95% CI −5.08 to 2.19); P=0.43), suggesting that the laughter exercise is not inferior to 0.1% sodium hyaluronic acid eyedrops (fig 2). The intention-to-treat analysis showed similar results (difference between the two groups was −1.32 points, 95% CI −4.90 to 2.26; P=0.47)(table 2). Both treatments were stopped at eight weeks at the trial end, with further evaluations made at 12 week. The laughter exercise group had a persistent and significantly greater decrease in the ocular surface disease index score than did the control group at 12 weeks (table 2, fig 2, supplementary figure 2): the mean between group difference was −4.08 points ((95% CI −7.62 to −0.55); P=0.024).

The secondary outcome of the proportion of participants whose ocular surface disease index score decreased by at least 10 points at eight weeks was 49.3 percentage points (95% CI 41.0 to 57.5) in the laughter exercise group versus 47.3 (39.3 to 55.3) in the 0.1% sodium hyaluronic acid group (mean difference 1.96 (95% CI −9.53 to 13.5); P≥0.05)(table 3). After eight weeks of treatments, the laughter exercise group had a more significant improvement in non-invasive tear break up time than did the control group, with a mean between group difference was 2.30 seconds ((95% CI 1.30 to 3.30); P<0.001) in the change from baseline to eight weeks (supplementary figure 3A). Changes in other secondary outcome measures (supplementary figure 3B-F) were not significant between the groups (all P≥0.05). As for the subjective scale scores, improvements were noted in the self-rating anxiety scale and self-rating depression scale score between baseline and eight weeks in both groups (all P<0.01 for the change in each group, but no significant change between groups), but not in the subjective happiness scale and Pittsburgh sleep quality index scores (table 3). Additionally, the laughter exercise group showed a significant improvement in the mental health subscale of the 36-item short form health survey after eight weeks (table 3).

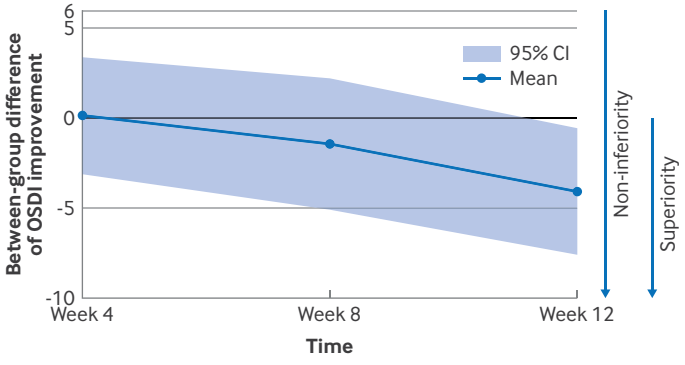

Fig 2 | The non-inferiority comparison between laughter exercise group and 0.1% sodium hyaluronic acid group. The upper bound of the 95% confidence interval (CI) −5.08 to 2.19 at eight weeks was less than the non-inferiority margin which had been prespecified as 6, indicating that laughter exercise was not inferior to 0.1% sodium hyaluronic acid at the primary end point, and tended to be converted to superiority at 12 weeks with the whole 95% CI below the zero line (−7.62 to −0.55). OSDI=ocular surface disease index

Discussion

Principal findings

Our results showed that laughter exercise was non-inferior to 0.1% sodium hyaluronic acid in alleviating dry eye disease symptoms. Additionally, we found that laughter exercise appeared to improve tear film stability and the meibomian gland function. We observed high

Table 2 | Adjusted effect of laughter exercise on primary outcome of OSDI score at eight weeks

Outcomes	Baseline*		Follow up		Change in OSDI†		Baseline adjusted between group difference in change in OSDI‡
	No.	Mean (SD)	No.	Mean (SD)	No.	Mean (95% CI)	Mean (95% CI)
Week 8 (end of treatment)							
Per protocol analysis (n=283):							
Laughter exercise group	136	35.6 (12.9)	136	25.1 (15.3)	136	−10.5 (−13.1 to −7.82)***	−1.45 (−5.08 to 2.19)
0.1% HA group	147	36.7 (14.5)	147	27.9 (17.7)	147	−8.83 (−11.7 to −6.02)***	P=0.43
Intention to treat analysis (n=299):							
Laughter exercise group	149	35.9 (13.6)	149	25.9 (15.9)	149	−10.0 (−12.8 to −7.29)***	−1.32 (−4.90 to 2.26)
0.1% HA group	150	36.8 (14.4)	150	28.0 (17.6)	150	−8.79 (−11.6 to −6.03)***	P=0.47
Week 12 (end of trial)							
Per protocol analysis (n=283):							
Laughter exercise group	137	35.9 (13.2)	137	21.3 (14.7)	137	−14.6 (−17.2 to −11.9)***	−4.08 (−7.62 to −0.55)
0.1% HA group	146	36.5 (14.2)	146	26.4 (17.3)	146	−10.1 (−13.0 to −7.30)***	P=0.02
Intention to treat analysis (n=299):							
Laughter exercise group	149	35.9 (13.6)	149	21.6 (15.1)	149	−14.3 (−17.1 to −11.5)***	−3.95 (−7.50 to −0.40)
0.1% HA group	150	36.8 (14.4)	150	26.5 (17.3)	150	−10.3 (−13.1 to −7.43)***	P=0.03

Data are mean (SD) or difference mean (95% CI).
CI=confidence interval; HA=hyaluronic acid; OSDI=ocular surface disease index; SD=standard deviation.
*No statistically significant differences between two groups for all baseline comparisons by two sample t-test.
†Paired t-test.
‡Linear regression with adjusting for OSDI grade at baseline.
***P<0.001.

cumulative compliance rates in the laughter exercise group.

Dry eye disease imposes an economic burden on both society and individuals because of healthcare use, such as repeated medical visits and long term eyedrops. Artificial tears are the mainstay of treatment for dry eye disease, and 0.1% sodium hyaluronic acid, one of the most widely used artificial tears, has a proven therapeutic effect in alleviating subjective ocular discomfort, and stabilising tear film in patients with dry eye disease. In terms of alleviating symptoms, laughter exercise was non-inferior to 0.1% sodium hyaluronic acid in the per protocol and intention-to-treat analyses in our study. The primary outcome was ocular surface disease index score, a well validated and widely used tool for outcome assessment in clinical trials of dry eye disease.[32 34] Moreover, laughter exercise significantly improved tear film stability and meibomian gland function. These benefits persisted for at least four weeks after discontinuation of the exercise, and such lasting efficacy was not noted in 0.1% sodium hyaluronic acid group.

Possible mechanisms

The biological mechanisms of laughter exercise on dry eye disease are unclear. Lacrimal gland, which secretes aqueous tears, and meibomian gland, which secretes lipid, are both innervated dominantly by parasympathetic nerves.[43] In the motor system, vocalisations in laughter involve the motoneuronal cell groups innervating the soft palate, pharynx, and larynx as well as the diaphragm, intercostal, abdominal, and pelvic floor muscles.[44] The contraction of respiratory muscles in laughter, especially abdominal breathing,[45] stimulates the autonomic nervous system including the activation of sympathetic and parasympathetic nervous system.[46 47] Consequently, laughter exercise stimulates tear secretion via autonomic nervous system activation.[48]

Notably, the contraction of orbicularis muscle (sphincter muscles of the eyelids) during laughter exercise is another plausible explanation. According to our results, patients in the laughter exercise group had greater improvements in the secretory capacity of the meibomian gland and properties of its secretion, when compared with 0.1% sodium hyaluronic acid. During the laughter exercise, patients were instructed to perform the vocalisations aloud and exaggerate their facial expressions, thus contracting the orbicularis oculi muscle, which function as the sphincter muscles of the eyelids, and are anatomically adjacent to the meibomian glands.[49] The contraction of the Riolan muscle, a distinct subdivision of striated orbicularis muscle, compresses the ductules of the meibomian gland, resulting in the extrusion of lipids onto the ocular surface.[50] Lipid tears are essential in maintaining tear film stability by delaying tear evapouration.[51]

Research has indicated that positive emotions in non-human animals can induce tear secretion through the release of oxytocin,[52] which could be triggered by laughter.[53] In our study, we hypothesised that positive emotions may also prompt tear secretion through deep breathing, relaxation, and laughter during the laughter exercise. The biological mechanisms underlying this effect is warranted further study.

Dry eye disease is not only a topical disease but also a systemic disease including brain and lifestyle.[11 54] Evidence indicates that lifestyle factors such as mental health can induce or modulate the severity of symptoms and signs of dry eye disease.[11 55 56] Symptoms of dry eye disease are closely associated with anxiety and depression.[11 57] Individuals with higher levels of subjective happiness have reported fewer symptoms of dry eye disease.[17 18] Laughter positively affects cognitive behaviour to improve and

Table 3 | Intention to treat analysis: unadjusted effect of laughter exercise on clinical signs of dry eye disease at eight weeks

	Laughter exercise group			0.1% hyaluronic acid group			Between group difference in change (95% CI)§
	Baseline†	Week 8	Change (95% CI)‡	Baseline†	Week 8	Change (95% CI)‡	
Secondary outcomes							
Proportion of decrease in OSDI total score of ≥10 (percentage points), (n=299 people)	NA	NA	49.3 (41.0 to 57.5)	NA	NA	47.3 (39.3 to 55.3)	1.96 (−9.53 to 13.5)
Clinical signs of dry eye disease, (n=598 eyes):							
Non-invasive tear break up time	7.95 (3.65)	11.2 (5.95)	3.21 (2.43 to 4.00)***	7.91 (3.69)	8.82 (4.38)	0.92 (0.29 to 1.54)**	2.30 (1.30 to 3.30)***
TMH (μm)	0.19 (0.05)	0.21 (0.06)	0.02 (0.01 to 0.03)***	0.19 (0.05)	0.20 (0.06)	0.005 (−0.002 to 0.010)	0.014 (0.002 to 0.025)
CFS score	0.76 (1.56)	0.57 (1.44)	−0.19 (−0.42 to 0.04)	0.59 (1.27)	0.53 (1.41)	−0.06 (−0.27 to 0.15)	−0.13 (−0.43 to 0.17)
Psychological outcomes, (n=299 people):							
SAS score	42.7 (9.15)	39.7 (10.1)	−3.05 (−4.39 to −1.71)***	41.3 (7.94)	39.3 (8.42)	−1.98 (−3.14 to −0.82)***	−1.07 (−2.84 to 0.70)
SDS score	45.8 (10.8)	42.8 (11.4)	−2.95 (−4.40 to −1.50)***	43.7 (10.4)	42.0 (11.1)	−1.68 (−2.95 to −0.41)**	−1.26 (−3.19 to 0.66)
SHS score	4.46 (1.24)	4.56 (1.18)	0.09 (−0.06 to 0.24)	4.42 (1.23)	4.52 (1.15)	0.10 (−0.05 to 0.25)	−0.003 (−0.21 to 0.21)
PSQI score	6.49 (3.01)	6.63 (2.90)	0.14 (−0.23 to 0.60)	6.67 (2.63)	6.84 (2.88)	0.17 (−0.25 to 0.59)	−0.04 (−0.66 to 0.59)
SF-36 score:							
Physical health subscale	92.6 (9.66)	94.0 (8.76)	1.46 (−0.04 to 2.95)	91.0 (11.6)	92.5 (11.2)	1.48 (0.11 to 2.84)*	−0.02 (−2.04 to 2.00)
Mental health subscale	64.7 (17.7)	68.0 (19.3)	3.28 (0.80 to 5.75)**	67.1 (16.3)	68.0 (17.0)	0.89 (−1.06 to 2.85)	2.39 (−0.78 to 5.55)
Exploratory outcomes (n=598 eyes)							
Fluorescein tear break-up time	1.89 (0.94)	3.70 (1.58)	1.82 (1.60 to 2.03)	1.91 (0.94)	2.23 (1.09)	0.32 (0.16 to 0.48)	1.50 (1.23 to 1.76)
Secretory capacity of meibomian gland	0.46 (0.68)	0.22 (0.47)	−0.24 (−0.33 to −0.14)	0.44 (0.64)	0.46 (0.64)	0.01 (−0.07 to 0.10)	−0.25 (−0.38 to −0.13)
Properties of meibomian glands secretion	2.20 (0.86)	1.84 (0.89)	−0.36 (−0.48 to −0.24)	2.22 (0.83)	2.35 (0.73)	0.13 (0.03 to 0.24)	−0.49 (−0.65 to −0.33)
Meibomian glands secretion property score	9.67 (5.29)	5.89 (4.17)	−3.78 (−4.45 to −3.12)	9.57 (4.94)	10.7 (4.84)	1.13 (0.54 to 1.71)	−4.91 (−5.80 to −4.02)

Data are mean (standard deviation) or difference mean (95% CI).
CFS=corneal fluorescein staining; CI=confidence interval; PSQI=Pittsburgh sleep quality index; SAS=self-rating anxiety scale; SDS=self-rating depression scale; SF-36=short form health survey; SHS=subjective happiness scale; TMH=tear meniscus height.
*P<0.05, **P<0.01, ***P<0.001, P value for between group difference was adjusted by the Benjamini-Hochberg with a false discovery rate of 0.05 for multiple comparisons. After adjustment, only the difference in non-invasive tear break-up time with P<0.001.
†No baseline difference between groups for clinical outcomes by generalised estimated equation (GEE) model with adjusting for intra-eye correlation, and two sample t-test for psychological outcomes.
‡GEE model with adjusting for intra-eye correlation for clinical signs and paired t-test for psychological outcomes.
§GEE model with adjusting for intra-eye correlation for clinical signs and two sample t-test for psychological outcomes.
For the exploratory outcomes (table 3), the laughter exercise group showed amelioration in fluorescein tear break-up time by 1.82 seconds (95% CI 1.60 to 2.03), better than that in 0.1% sodium hyaluronic acid group (mean difference 1.50 seconds (95% CI 1.23 to 1.76)). Moreover, the function of the meibomian gland was improved only in the laughter exercise group, with the meibomian gland secretory capacity improved by −0.24 (95% CI −0.33 to −0.14), and meibomian gland secretion property score improved by −3.78 (−4.45 to −3.12). The above clinical signs for monocular results were consistent with those for binoculars (supplementary table 1).
No adverse events were reported in either study group.

establish healthy physical, psychological, and social relationships, and thereby quality of life.[58-60] A study published in 2022 indicated that a facial mimicry laughter could both amplify and initiate feelings of happiness, regardless of the presence or absence of emotional stimuli.[61] We modified our laughter exercise by facial mimicry and voluntary facial action tasks, which might have a similar positive effect to laughter.[61] Evidence suggests that laughter therapy is a tool in lifestyle medicine that can improve sleep quality, and physical and psychological functions (eg, body weight, subjective stress, subjective well being),[62] and promote energy expenditure.[63-65] We also observed the improved mental health score in laughter exercise group. More frequent daily laughter is associated with a lower prevalence of lifestyle related diseases, such as hypertension, diabetes mellitus, and heart disease.[66] Thus, laughter exercise might alleviate dry eye disease indirectly by creating a persisting positive effect on lifestyle. This effect might also explain the continued improvement in ocular surface disease index observed between eight and 12 weeks, following the termination of the trial.

Strengths and limitations of this study

Strengths of this trial include the randomised controlled design, careful compliance monitoring and support, high rates of both compliance and follow-up, and good effort to standardise the laughter exercise intervention with the use of videos.

However, our study had limitations. A double blinded study design was not practical because this would necessitate a sham laughter exercise for which no approach has been validated. To minimise possible placebo effects, participants were informed that the study aimed to compare the effects of two different interventions without alluding to the possible effects of laughter exercise. Additionally, laughter exercise has a greater time investment when compared with the use of eyedrops, albeit minimal.

Future directions

Future research should assess the most effective frequency and duration of laughter exercise. The biological mechanisms warrant further testing by experimental studies. Furthermore, research into laughter exercise for other ocular conditions compared with other artificial tears, with or without lipids, and

higher concentrations of sodium hyaluronic acid, would provide a more comprehensive understanding of therapeutic potential. Moreover, the association between dry eye disease and lifestyle disorders is complicated, and a comprehensive approach should be investigated in the future.

Conclusions

The findings of this randomised controlled trial suggest that laughter exercise, four times a day, was non-inferior to 0.1% sodium hyaluronic acid, four times a day, in improving dry eye disease symptoms and clinical signs. As a safe, environmentally friendly, and low cost intervention, laughter exercise could serve as a first-line, home based treatment for people with symptomatic dry eye disease and limited corneal staining.

Contributors: JL, YLia, and S-YZ contributed equally to the study and are joint first authors. LL, ZL, and YLiu conceived and designed the study. LL, JL, YLia, S-YZ, LJ, ZF, ZL, YLiu conducted the study, including acquisition, analysis, or interpretation of data. LJ and NC were responsible for statistical analysis. JL, LL, LJ, and NC drafted the manuscript. JL and LL obtained funding. LL, JL, LJ, NC, YZe, YZh, ZL, and YLiu were responsible for administrative, technical, or material support. LL, YZe, YZh, YLiu, and ZL were responsible for study supervision. All authors critically revised the manuscript. All authors gave final approval of the manuscript. LL, YLiu, and ZL contributed equally to this work and are the joint corresponding authors. LL is the guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Ethical approval: Ethical approval was obtained from the Ethics Committee of Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China (2020KYPJ010). Written informed consent was obtained from all participants.

Data sharing: All data requests should be submitted to lianglingyi@gzzoc.com for consideration. Access to anonymised data may be granted after review.

Patient consent: Patient consent obtained.

Transparency: The manuscript's guarantor (LL) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as originally planned have been explained.

Dissemination to participants and related patient and public communities: A link to the publication will be sent to the research participants. The main findings of this trial will be disseminated to clinicians, patients and to the public via press releases, social media, and educational and training materials; presented to the scientific community at academic conferences; and shared with clinicians caring for patients with dry eye disease.

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1 Clayton JA. Dry eye. *N Engl J Med* 2018;378:2212-23. doi:10.1056/NEJMra1407936
2 Craig JP, Nelson JD, Azar DT, et al. TFOS DEWS II report executive summary. *Ocul Surf* 2017;15:802-12. doi:10.1016/j.jtos.2017.08.003
3 Tsubota K, Yokoi N, Shimazaki J, et al,Asia Dry Eye Society. New perspectives on dry eye definition and diagnosis: a consensus report by the Asia dry eye society. *Ocul Surf* 2017;15:65-76. doi:10.1016/j.jtos.2016.09.003
4 2022 Market Scope. Dry eye products report: a global market analysis for 2015 to 2021. St Louis: Market Scope. November, 2022.
5 Sullivan DA, Rocha EM, Aragona P, et al. TFOS DEWS II Sex, Gender, and Hormones Report. *Ocul Surf* 2017;15:284-333. doi:10.1016/j.jtos.2017.04.001
6 Straits Research. Global report of artificial tears market: information by type (cellulose derived tears, glycerin derived tears), applications (dry eyes treatment, and contact lenses moisture), and regions-forecast till 2031. Report Code: SRTE438DR. 2019. https://straitsresearch.com/report/artificial-tears-market.
7 Yu J, Asche CV, Fairchild CJ. The economic burden of dry eye disease in the United States: a decision tree analysis. *Cornea* 2011;30:379-87. doi:10.1097/ICO.0b013e3181f7f363
8 Galor A, Feuer W, Lee DJ, et al. Depression, post-traumatic stress disorder, and dry eye syndrome: a study utilizing the national United States Veterans Affairs administrative database. *Am J Ophthalmol* 2012;154:340-346.e2. doi:10.1016/j.ajo.2012.02.009
9 van der Vaart R, Weaver MA, Lefebvre C, Davis RM. The association between dry eye disease and depression and anxiety in a large population-based study. *Am J Ophthalmol* 2015;159:470-4. doi:10.1016/j.ajo.2014.11.028
10 Kim KW, Han SB, Han ER, et al. Association between depression and dry eye disease in an elderly population. *Invest Ophthalmol Vis Sci* 2011;52:7954-8. doi:10.1167/iovs.11-8050
11 Galor A, Britten-Jones AC, Feng Y, et al. TFOS Lifestyle: Impact of lifestyle challenges on the ocular surface. *Ocul Surf* 2023;28:262-303. doi:10.1016/j.jtos.2023.04.008
12 Galor A. How depression might relate to dry eye disease. *JAMA Ophthalmol* 2022;140:399-400. doi:10.1001/jamaophthalmol.2022.0146
13 Ong ES, Felix ER, Levitt RC, Feuer WJ, Sarantopoulos CD, Galor A. Epidemiology of discordance between symptoms and signs of dry eye. *Br J Ophthalmol* 2018;102:674-9. doi:10.1136/bjophthalmol-2017-310633
14 Kuang TM, Tsai SY, Liu CJ, Lee SM, Chou P. Association between dry eye and depressive symptoms in an elderly Chinese population in Taiwan: the Shihpai Eye Study. *Eye (Lond)* 2021;35:2826-33. doi:10.1038/s41433-020-01329-5
15 Inomata T, Iwagami M, Nakamura M, et al. Association between dry eye and depressive symptoms: Large-scale crowdsourced research using the DryEyeRhythm iPhone application. *Ocul Surf* 2020;18:312-9. doi:10.1016/j.jtos.2020.02.007
16 WHO South-East Asia. Promotion of mental well-being: pursuit of happiness. *World Health Organization* 2013.
17 Negishi K, Ayaki M, Kawashima M, Tsubota K. Sleep and subjective happiness between the ages 40 and 59 in relation to presbyopia and dry eye. *PLoS One* 2021;16:e0250087. doi:10.1371/journal.pone.0250087
18 Kawashima M, Uchino M, Yokoi N, et al. Associations between subjective happiness and dry eye disease: a new perspective from the Osaka study. *PLoS One* 2015;10:e0123299. doi:10.1371/journal.pone.0123299
19 Cousins N. Anatomy of an illness (as perceived by the patient). *N Engl J Med* 1976;295:1458-63. doi:10.1056/NEJM197612232952605
20 Ljungdahl L. Laugh if this is a joke. *JAMA* 1989;261:558. doi:10.1001/jama.1989.03420040092022
21 van der Wal CN, Kok RN. Laughter-inducing therapies: Systematic review and meta-analysis. *Soc Sci Med* 2019;232:473-88. doi:10.1016/j.socscimed.2019.02.018
22 Akimbekov NS, Razzaque MS. Laughter therapy: A humor-induced hormonal intervention to reduce stress and anxiety. *Curr Res Physiol* 2021;4:135-8. doi:10.1016/j.crphys.2021.04.002
23 Bressington D, Mui J, Yu C, et al. Feasibility of a group-based laughter yoga intervention as an adjunctive treatment for residual symptoms of depression, anxiety and stress in people with depression. *J Affect Disord* 2019;248:42-51. doi:10.1016/j.jad.2019.01.030
24 Martin RA. Humor, laughter, and physical health: methodological issues and research findings. *Psychol Bull* 2001;127:504-19. doi:10.1037/0033-2909.127.4.504
25 Öztürk FO, Bayraktar EP, Tezel A. The effect of laughter yoga on loneliness, psychological resilience, and quality of life in older adults: a pilot randomized controlled trial. *Geriatr Nurs* 2023;50:208-14. doi:10.1016/j.gerinurse.2023.01.009

26 Kiyak S, Kocoglu-Tanyer D. Effectiveness of progressive muscle relaxation and laughter therapy on mental health and treatment outcomes in women undergoing in vitro fertilization: A randomized controlled trial. *Res Nurs Health* 2021;44:945-56. doi:10.1002/nur.22187
27 Stiwi K, Rosendahl J. Efficacy of laughter-inducing interventions in patients with somatic or mental health problems: A systematic review and meta-analysis of randomized-controlled trials. *Complement Ther Clin Pract* 2022;47:101552. doi:10.1016/j.ctcp.2022.101552
28 Shi H, Wu Y, Wang L, Zhou X, Li F. Effects of laughter therapy on improving negative emotions associated with cancer: a systematic review and meta-analysis. *Oncology* 2024;102:343-53. doi:10.1159/000533690
29 Kumar VR, Mohan MVR, Kumar GV, Kalra S. Laughter therapy in diabetes. *J Pak Med Assoc* 2021;71:1696-7.
30 Hirosaki M, Ohira T, Wu Y, et al. Laughter yoga as an enjoyable therapeutic approach for glycemic control in individuals with type 2 diabetes: A randomized controlled trial. *Front Endocrinol (Lausanne)* 2023;14:1148468. doi:10.3389/fendo.2023.1148468
31 Kiviniemi MT, Voss-Humke AM, Seifert AL. How do I feel about the behavior? The interplay of affective associations with behaviors and cognitive beliefs as influences on physical activity behavior. *Health Psychol* 2007;26:152-8. doi:10.1037/0278-6133.26.2.152
32 Wolffsohn JS, Arita R, Chalmers R, et al. TFOS DEWS II diagnostic methodology report. *Ocul Surf* 2017;15:539-74. doi:10.1016/j.jtos.2017.05.001
33 Subcommittee of the International Dry Eye WorkShop, The Ocular Surface. Methodologies to diagnose and monitor dry eye disease: report of the Diagnostic Methodology Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf* 2007;5:108-52. doi:10.1016/S1542-0124(12)70083-6
34 Asbell PA, Maguire MG, Pistilli M, et al,Dry Eye Assessment and Management Study Research Group. N-3 fatty acid supplementation for the treatment of dry eye disease. *N Engl J Med* 2018;378:1681-90. doi:10.1056/NEJMoa1709691
35 Zhang Y, Qu B, Lun SS, Guo Y, Liu J. The 36-item short form health survey: reliability and validity in Chinese medical students. *Int J Med Sci* 2012;9:521-6. doi:10.7150/ijms.4503
36 Liu XC, Oda S, Peng X, Asai K. Life events and anxiety in Chinese medical students. *Soc Psychiatry Psychiatr Epidemiol* 1997;32:63-7. doi:10.1007/BF00788922
37 Liu XC, Ma DD, Kurita H, Tang MQ. Self-reported depressive symptoms among Chinese adolescents. *Soc Psychiatry Psychiatr Epidemiol* 1999;34:44-7. doi:10.1007/s001270050110
38 Tsai PS, Wang SY, Wang MY, et al. Psychometric evaluation of the Chinese version of the Pittsburgh Sleep Quality Index (CPSQI) in primary insomnia and control subjects. *Qual Life Res* 2005;14:1943-52. doi:10.1007/s11136-005-4346-x
39 Nan H, Ni MY, Lee PH, et al. Psychometric evaluation of the Chinese version of the Subjective Happiness Scale: evidence from the Hong Kong FAMILY Cohort. *Int J Behav Med* 2014;21:646-52. doi:10.1007/s12529-014-9389-3
40 Royston P. Multiple imputation of missing values. *Stata J* 2004;4:227-41. doi:10.1177/1536867X0400400301.
41 Schaumberg DA, Sullivan DA, Buring JE, Dana MR. Prevalence of dry eye syndrome among US women. *Am J Ophthalmol* 2003;136:318-26. doi:10.1016/S0002-9394(03)00218-6
42 Schaumberg DA, Dana R, Buring JE, Sullivan DA. Prevalence of dry eye disease among US men: estimates from the Physicians' Health Studies. *Arch Ophthalmol* 2009;127:763-8. doi:10.1001/archophthalmol.2009.103
43 Dartt DA. Neural regulation of lacrimal gland secretory processes: relevance in dry eye diseases. *Prog Retin Eye Res* 2009;28:155-77. doi:10.1016/j.preteyeres.2009.04.003
44 Holstege G, Subramanian HH. Two different motor systems are needed to generate human speech. *J Comp Neurol* 2016;524:1558-77. doi:10.1002/cne.23898
45 Sano K, Kawashima M, Ikeura K, Arita R, Tsubota K. Abdominal breathing increases tear secretion in healthy women. *Ocul Surf* 2015;13:82-7. doi:10.1016/j.jtos.2014.08.002
46 Giuliani NR, McRae K, Gross JJ. The up- and down-regulation of amusement: experiential, behavioral, and autonomic consequences. *Emotion* 2008;8:714-9. doi:10.1037/a0013236
47 Sakuragi S, Sugiyama Y, Takeuchi K. Effects of laughing and weeping on mood and heart rate variability. *J Physiol Anthropol Appl Human Sci* 2002;21:159-65. doi:10.2114/jpa.21.159
48 Belmonte C, Nichols JJ, Cox SM, et al. TFOS DEWS II pain and sensation report. *Ocul Surf* 2017;15:404-37. doi:10.1016/j.jtos.2017.05.002
49 Black DW. Laughter. *JAMA* 1984;252:2995-8. doi:10.1001/jama.1984.03350210043027
50 Knop E, Knop N, Millar T, Obata H, Sullivan DA. The international workshop on meibomian gland dysfunction: report of the subcommittee on anatomy, physiology, and pathophysiology of the meibomian gland. *Invest Ophthalmol Vis Sci* 2011;52:1938-78. doi:10.1167/iovs.10-6997c
51 Willcox MDP, Argüeso P, Georgiev GA, et al. TFOS DEWS II Tear Film Report. *Ocul Surf* 2017;15:366-403. doi:10.1016/j.jtos.2017.03.006
52 Murata K, Nagasawa M, Onaka T, et al. Increase of tear volume in dogs after reunion with owners is mediated by oxytocin. *Curr Biol* 2022;32:R869-70. doi:10.1016/j.cub.2022.07.031
53 Pfundmair M. Finding humor in hormones: oxytocin promotes laughing and smiling. *Biol Psychol* 2022;172:108377. doi:10.1016/j.biopsycho.2022.108377
54 Kaido M, Arita R, Mitsukura Y, Tsubota K. Electroencephalogram-detected stress levels in the frontal lobe region of patients with dry eye. *Ocul Surf* 2024;32:139-44. doi:10.1016/j.jtos.2024.02.007
55 Kawashima M, Yamada M, Shigeyasu C, et al. Association of systemic comorbidities with dry eye disease. *J Clin Med* 2020;9:2040. doi:10.3390/jcm9072040
56 Hanyuda A, Sawada N, Uchino M, et al. Relationship between unhealthy sleep status and dry eye symptoms in a Japanese population: The JPHC-NEXT study. *Ocul Surf* 2021;21:306-12. doi:10.1016/j.jtos.2021.04.001
57 Stapleton F, Alves M, Bunya VY, et al. TFOS DEWS II epidemiology report. *Ocul Surf* 2017;15:334-65. doi:10.1016/j.jtos.2017.05.003
58 Kuru N, Kublay G. The effect of laughter therapy on the quality of life of nursing home residents. *J Clin Nurs* 2017;26:3354-62. doi:10.1111/jocn.13687
59 Heidari M, Ghodusi Borujeni M, Rezaei P, Kabirian Abyaneh S, Heidari K. Effect of laughter therapy on depression and quality of life of the elderly living in nursing homes. *Malays J Med Sci* 2020;27:119-29. doi:10.21315/mjms2020.27.4.11
60 Tamada Y, Takeuchi K, Yamaguchi C, et al. Does laughter predict onset of functional disability and mortality among older japanese adults? The JAGES prospective cohort study. *J Epidemiol* 2021;31:301-7. doi:10.2188/jea.JE20200051
61 Coles NA, March DS, Marmolejo-Ramos F, et al. A multi-lab test of the facial feedback hypothesis by the Many Smiles Collaboration. *Nat Hum Behav* 2022;6:1731-42. doi:10.1038/s41562-022-01458-9
62 Louie D, Brook K, Frates E. The laughter prescription: a tool for lifestyle medicine. *Am J Lifestyle Med* 2016;10:262-7. doi:10.1177/1559827614550279
63 Harputlu D, Öztürk FO, Aydın D, Akyol C, Tezel A. Effect of laughter yoga on sleep and quality of life in individuals with fecal ostomies: a randomized controlled trial. *J Wound Ostomy Continence Nurs* 2023;50:307-12. doi:10.1097/WON.0000000000000988
64 Funakubo N, Eguchi E, Hayashi R, et al. Effects of a laughter program on body weight and mental health among Japanese people with metabolic syndrome risk factors: a randomized controlled trial. *BMC Geriatr* 2022;22:361. doi:10.1186/s12877-022-03038-y
65 Buchowski MS, Majchrzak KM, Blomquist K, Chen KY, Byrne DW, Bachorowski JA. Energy expenditure of genuine laughter. *Int J Obes (Lond)* 2007;31:131-7. doi:10.1038/sj.ijo.0803353
66 Eguchi E, Ohira T, Nakano H, et al,On Behalf Of The Fukushima Health Management Survey Group. Association between laughter and lifestyle diseases after the great east japan earthquake: The Fukushima health management survey. *Int J Environ Res Public Health* 2021;18:12699. doi:10.3390/ijerph182312699

Web appendix: Extra material supplied by authors
Web appendix: Patient Instructions for the Laughter Exercise
Web appendix: Study protocol

Video 1: Instructions given to the laughter exercise group translated into English
Video 2: Instructions given to the laughter exercise group in Mandarin Chinese video: "训练过程中, 请尽量夸张表情, 上扬嘴角, 露出牙齿, 眯起眼睛。请跟我念:"Hihihi (用普通话拼音拼读),哈哈哈,茄子茄子茄子,七喜七喜七喜,哈哈哈哈哈哈"。以上为一遍口令,每一次重复30遍,一天训练4次。