# Influence of a Controlled Environment Simulating an In-Flight Airplane Cabin on Dry Eye Disease

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**Purpose.** To evaluate symptoms, signs, and the levels of 16 tears inflammatory mediators of dry eye (DE) patients exposed to an environment simulating an in-flight air cabin in an environmental chamber.

**Methods.** Twenty DE patients were exposed to controlled environment simulating an in-flight airplane cabin (simulated in-flight condition [SIC]) of 23°C, 5% relative humidity, localized air flow, and 750 millibars (mb) of barometric pressure. As controls, 15 DE patients were subjected to a simulated standard condition (SSC) of 23°C, 45% relative humidity, and 930 mb. A DE symptoms questionnaire, diagnostic tests, and determination of 16 tear molecules by multiplex bead array were performed before and 2 hours after exposure.

**Results.** After SIC exposure, DE patients became more symptomatic, suffered a significant ($P \leq 0.05$) decrease in tear stability (tear break up time) (from 2.18 ± 0.28 to 1.53 ± 0.20), and tear volume (phenol red thread test), and a significant ($P \leq 0.05$) increase in corneal staining, both globally (0.50 ± 0.14 before and 1.25 ± 0.19 after) and in each area (Baylor scale). After SSC, DE patients only showed a mild, but significant ($P \leq 0.05$), increase in central and inferior corneal staining. Consistently, tear levels of IL-6 and matrix metalloproteinase (MMP)-9 significantly increased and tear epidermal growth factor (EGF) significantly decreased ($P \leq 0.05$) only after SIC.

**Conclusions.** The controlled adverse environment conditions in this environmental chamber can simulate the conditions in which DE patients might be exposed during flight. As this clearly impaired their lacrimal functional unit, it would be advisable that DE patients use therapeutic strategies capable of ameliorating these adverse episodes. (*Invest Ophthalmol Vis Sci.* 2013;54:2093–2099) DOI:10.1167/iovs.12-11361

Dry eye (DE) is a common disorder affecting millions of people worldwide. The prevalence of this disease varies depending on the criteria used for diagnosis.[1] It is a multifactorial disease of the lacrimal functional unit (LFU)[2] characterized by ocular discomfort and pain, visual disturbance, tear film instability, increased tear osmolarity, and inflammation.[2] Symptoms and signs can greatly vary depending upon the environmental conditions to which patients are exposed in their daily lives. These environmental variables mainly include temperature, wind, humidity, presence of localized air flow, atmospheric pressure, and exposure to pollutants. Currently, millions of individuals are exposed to adverse environments that are artificially controlled such as air conditioned rooms (creating a global disorder known as sick building syndrome),[3] vehicles, or airplane cabins. For instance, a total of 777 million passengers were carried by air in 2011 only in the EU-27 countries (according to EUROSTAT statistics); while 707 million were in the United States of America (USA), according to the International Civil Aviation Organization statistics; hence, a large number of people worldwide affected by DE syndrome[4] are exposed daily to these adverse conditions.

Airplanes cabins are perfect examples of controlled indoor environment. Low humidity within the cabin is caused by the need for continuous renewal of air coming from the outside. This air is characterized by low humidity as a result of the low temperature (–43°C [–45°F] to –65°C [–85°F]) typically found at 10,660 m (35,000 ft), which is usually reached during a transatlantic flight.[5] Thus, humidity within a flight cabin has been reported to range from 5% to 25%.[6] Additionally, the barometric pressure is also reduced, ranging from 810 to 750 millibars (mb) corresponding to an altitude ranging from 1830 m (6000 ft) to 2440 m (8000 ft) above sea level,[7] while temperature usually varies from 21°C (70°F) to 25°C (77°F).[6] To maintain overall cabin temperature control and cabin pressurization high air exchange rates are necessary resulting in an increased air flow.[5] As these controlled indoor environments are currently inevitable, it is essential to define how these conditions can affect individuals in order to design strategies that can protect susceptible people from developing disease or from worsening their previous disease (i.e., DE patients).

The purpose of the present study is to analyze how the LFU of DE patients is affected after exposure to a controlled adverse environment similar to the one usually found within an airplane cabin. This condition was created in an environmental chamber and the effects of that environment were evaluated by

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DE-related symptoms, signs, and the concentration of 16 inflammatory mediators, tested as potential biomarkers.

## Materials and Methods

### Participants

The study protocols adhered to the tenets of the Declaration of Helsinki and were approved by the University of Valladolid ethics committee. The study was double masked and the same examiner always performed the clinical subjective tests.

DE patients were recruited among Level 1 and 2, as classified by the International Dry Eye Workshop (DEWS) DE severity grading scheme.[1] These patients were already being cared for at the Ocular Immunology Unit of IOBA (University of Valladolid) and were regularly performing daily palpebral hygiene and using non-preserved artificial and/or ocular lubricants as the only topical medication.

Inclusion criteria were an ocular surface disease index (OSDI)[8] score greater than 12, corneal staining less than or equal to grade 2 (Oxford Scale),[9] and lid hygiene and non-preserved artificial tears as the only topical medication. Exclusion criteria were an age younger than 40 years, contact lens wear, pregnancy or nursing, any ocular surgery within the last 6 months, any acute or chronic ocular disease other than DE, concomitant allergies (even if mild), topical medications (only artificial tears were allowed), and any systemic anomaly that contraindicated being subjected to any environmental controlled condition. Use of systemic medication had to be unchanged during the preceding 3 months and for the entire duration of the study. Patients were instructed not to instill their artificial tears within the 4 hours prior to evaluation.

The eye having higher corneal staining was selected for the study; if both eyes were similar, then the most symptomatic eye was chosen; otherwise, the study eye was selected using a random table.

### Environmental Chamber and Environmental Conditions

Individuals were exposed to two different environment conditions within an environmental chamber (VISIÓN I+D, SL; Valladolid, Spain) inside the Controlled Environment Research Laboratory (CERLab), located at the Institute for Applied OphthalmoBiology (IOBA) building at the University of Valladolid, Valladolid, Spain. This facility is composed of an exposure chamber and an evaluation chamber. Temperature (range: 15–30°C, 1°C steps) and relative humidity (RH, range: 5%–80%, 1% steps) can be controlled in both rooms simultaneously. Additionally, airflow (blower exit velocity: range 0.60–3.60 m/s), illumination (range, 10–1000 lux, 1 lux steps) and atmospheric pressure [range: 930–450 mb, 1 mb steps] can also be controlled in the exposure chamber. Using a control display located outside, environmental conditions within the chamber were monitored throughout the entire duration of the experiments and were recorded in 5 minute intervals. Experimental sessions were run only when an anesthesiologist working at the surgery facilities (located the next room) was also available to take care of any possible medically adverse event.

Between 2 and 15 days after the screening visit, participants were randomly assigned to one of the two following environmental conditions: (1) a simulated in-flight condition (SIC) of 23°C, 5% RH, localized air flow (individual blowers located 1 m [39.37 inches] away from each subject; mean velocity of 0.43 m/s), and 750 mb of barometric pressure (similar to that usually found within an aircraft cabin at 2500 m [8200 ft] above sea level).[10] Pressure changes were designed to simulate an actual aircraft flight, with a 15 minute ascent during which the pressure decreased at a rate of 12.7 mb/min, an 85 minute cruise, and a 20 minute descent during which the pressure increased at a rate of 9.5 mb/min. Participants watched a documentary on a light-emitting diode television monitor of 55 inches (LG Electronics Inc., Gumi, South Korea) situated above eye level, or (2) a simulated standard condition (SSC) of 23°C, 45% RH, 930 mb (average atmospheric pressure in Valladolid, which is 690 m above sea level), and participants performed near vision tasks (reading, playing cards, etc).

### Examination Procedure

Recruited patients were evaluated in the examination chamber twice: immediately before and immediately after the 2 hour period of SIC and SSC exposure. The examinations were performed in the sequence outlined below, with a 2 to 5 minute interval between tests. Subjective tests were always performed by the same examiner.

**Modified Single-Item Score Dry Eye Questionnaire (SIDEQ).** This questionnaire assesses the ocular discomfort due to symptoms of dryness, ranging from "none" to "severe" (0–4 scale) (Simmons PA. *IOVS* 2003;44:ARVO E-Abstract B287); however, we additionally included a visual analogue scale (VAS)[11] to increase test sensitivity. The VAS consists of a horizontal line 100-mm long. At the left end of the line the number "0" indicated the absence of symptoms. At the right end of the line, the number "100" indicated severe symptoms.

**Tear Osmolarity.** Tear osmolarity was assessed using the TearLab (TearLab Corporation, San Diego, CA), which only needs a 50 nL tear sample from the external canthus to provide osmolarity values. The recommended cutoff value for DE diagnosis is 316 mOsm/L.[12,13]

**Phenol Red Thread Test (PRTT).** The PRTT was acquired by Menicon Company Ltd. (Zone Quick Test; Nagoya, Japan). The thread was placed over the external canthus, and the length of the wetted thread was read 15 seconds later.[14] Values of 20 mm or below were considered abnormal.[15]

**Conjunctival Bulbar Hyperemia.** Nasal and temporal areas were assessed independently based on the Efron scale[16] and the final score was obtained after averaging both values.

**Tear Sample Collection for Molecule Analyses.** As previously described,[17] 2 μL were collected from the external canthus using a glass capillary tube (Drummond Scientific, Broomall, PA) and in a non-traumatic manner to avoid as much as possible reflex tear secretion.

**Fluorescein Tear Break-Up Time (T-BUT).** After instillation of 5 μL of 2% sodium fluorescein, the time between the last of three blinks and the appearance of the first dry spot was measured three times and the mean value was recorded. Values of less than or equal to 7 seconds are currently considered abnormal.[18]

**Corneal Fluorescein Staining (CFS).** CFS was evaluated 2 minutes after instillation of 5 μL of 2% sodium fluorescein and using a cobalt blue filter (Topcon Corp., Tokyo, Japan) over the light source slit-lamp biomicroscope (SL-8Z; Topcon Corp.) and a yellow Wratten #12 filter (Eastman Kodak, Rochester, NY). The Oxford scheme (0–5 score)[19] and a slightly modified Baylor scheme[20] were used. The later assesses the CFS by dividing the cornea into central, superior, temporal, inferior, and nasal zones.[20] Grading the intensity of the staining for each zone is based on the number of dots found on a 5 point scale (0 = no dots; 4 = above 30 dots). Moreover, the Baylor scheme requires adding 1 point to the final score if there is one area of confluence, and 2 points if there are two or more areas of confluence or there is filamentary keratitis. With the aim of providing a more detailed CFS analysis we simply split the grading scheme into the two scales that are originally added up; therefore, we first graded intensity in each corneal area, and second, we graded severity (presence of confluence and/or filamentary keratitis). We always used a customized template incorporated to the biomicroscope viewing system to increase grading accuracy.

**Conjunctival Lissamine Green Staining.** Lissamine green strips (GreenGlo; HUB Pharmaceuticals, LLC, Rancho Cucamonga, CA) were wetted with 25 μL sodium chloride and then gently applied into the inferior fornix. One minute later, staining was evaluated following the Oxford scheme.[19]

**Schirmer Test without Topical Anesthesia.** One Schirmer sterile strip (Tearflo, HUB Pharmaceuticals, LLC) was placed in the lateral canthus of the inferior lid margin.[21] The length of wetting was measured after 5 minutes, with eyes closed. Results of 5-mm length or more were considered normal.

## Analysis of Tear Molecules

Samples (2 μL) were diluted 1:10 in a 0.5-mL microtube (Sarstedt AG&Co, Nümbrecht, Germany) containing Cytokine Assay Buffer (Merck Millipore, Millipore Iberica, Madrid, Spain) and frozen at −80°C until analysis. A commercial immune-bead based assays in a Luminex IS-100 (Luminex Corporation, Austin, TX) was used to analyze 16 molecules. The concentrations of epidermal growth factor (EGF), CX3CL1/fractalkine, interferon (IFN)-γ, IL-10, IL-12p70, IL-17A, IL-1β, IL-1RA, IL-2, IL-6, CXCL8/IL-8, CXCL10/IP-10, CCL5/RANTES, TNF-α, and VEGF were measured simultaneously with a 15-plex assay (HCYTO-60K 15X-Milliplex, Millipore Iberica, Madrid, Spain). Matrix metalloproteinase (MMP)-9 concentration was measured in a separate assay with a MMP-9 single-plex assay (HMMP2-55K Panel 2; Milliplex, Millipore Iberica). The samples were analyzed following the manufacturer's protocol. Briefly, 10 μL of the 1:10 diluted sample were incubated under agitation overnight at 4°C with beads coated with antibodies specific for each molecule. After washing, beads were incubated with biotinylated human antibodies for 1 hour, followed by incubation with streptavidin-phycoerythrin for 30 minutes. Standard curves were used to convert fluorescence units to concentration units (pictograms/milliliter). The minimum detectable concentrations (in pictograms/milliliter) for molecules analyzed were IFN-γ and TNF-α, 0.1; CXCL8/ IL-8 and IL-17A, 0.2; IL-2, IL-6, and IL-10, 0.3; IL-1β, and IL-12p70, 0.4; CCL5/RANTES, 1; CXCL10/IP-10, 1.2; EGF, 2.7; IL-1RA, 2.9; VEGF, 5.8; CX3CL1/Fractalkine, 6; and MMP-9, 10. Data were stored and analyzed with the Bead View Software (Upstate-Millipore Corporation, Watford, UK). In some samples, the assayed molecule was undetectable. To include those samples in the statistical analysis, we assigned each the minimum detectable value (provided by the assay manufacturer). However, molecules that were detected in less than 30% of the samples were not further analyzed.

## Data Analysis

Data were expressed as the mean ± SEM. Statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS 18.0 for Windows; SPSS Inc., Chicago, IL) and R software by a licensed statistician (co-author MEM). The Mann-Whitney *U* test was used for comparisons of two independent sample groups. For comparisons between tests performed prior to and after the environmental exposure, the Wilcoxon signed rank test was used for quantitative variables and McNemar test for qualitative variables. Two-sided *P* values less than or equal to 0.05 were considered statistically significant.

# Results

## Screening Visit

A total of 20 (6 males, 14 females; 64.7 ± 1.8-years-old) and 15 (5 males, 10 females; 60.3 ± 2.1-years-old) DE patients were included for the SIC and the SSC environmental conditions, respectively. Screening evaluation outcomes obtained for each DE groups were not significantly different (Table 1).

## Clinical Tests

As shown in Table 1, none of the values regarding the pre-exposure visits were statistically different between the two groups. Both groups were similarly symptomatic, had osmolarity values above the most recent recommended cutoff value[12,13] and also shared quite low BUT values. Vital staining was negative or minimal, as corresponds to the level 1 DE patients that we meant to recruit.

When both groups of DE patients exited the environmental chamber after a 2 hour exposure to either SIC or SSC, there were some variations, shown in Table 2. The modified SIDEQ global scores increased after SIC exposure close to statistical significance; additionally, burning and itching sensation significantly increased after SIC ($P \leq 0.05$).

Conjunctival hyperemia slightly but significantly increased in all individuals after both exposures, while staining was negligible before and after exposure.

Corneal staining scored by the more global Oxford method increased significantly only after the SIC. The more segmented and detailed Baylor scheme also showed an increased number of dots (intensity scale) in all five areas. Likewise, severity of CFS was significantly increased only after the SIC in the inferior area. SSC on the other hand, provoked a significantly greater CFS only in central and inferior areas.

Regarding those tests measuring tear quantity, Schirmer was unmodified, but PRTT scores were significantly decreased after SIC and lower than those shown after SSC.

Finally, tear stability as evaluated by T-BUT significantly decreased only under SIC exposure, this value being also lower than that showed by DE subjects after SSC. Tear osmolarity did not vary significantly after any of the two conditions, although the value after SIC was significantly higher than the one showed after SSC. No individuals suffered any adverse event after any exposure.

## Tear Molecule Levels

Among the 16 molecules analyzed in tears, CX3CL1/Fractalkine, EGF, CXCL8/IL-8, IL-1RA, CXCL10/IP-10, and MMP-9 were detected in more than 90% of DE patients of either group, both before and after exposure. IL-6, CCL5/RANTES, and VEGF were detected in 58% to 80% in both groups prior to entering the environmental chamber and in 69% to 90% after exposure to either environment. The detection rates for IFN-γ, IL-12p70, IL-17, IL-10, IL-1β, and TNF-α were below 30% for both groups under both exposure conditions, and were therefore not considered for further analysis (see Supplementary Material and Supplementary Table S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-11361/-/DCSupplemental for tear molecule concentrations).

**Table 1.** Screening Evaluation of DE Patients Randomized for the Two Environmental Conditions: SIC and SSC

| Diagnostic Tests | SIC | SSC | *P* Value* |
|---|---|---|---|
| OSDI score | 35.73 ± 2.79 | 40.60 ± 5.97 | 0.90 |
| Phenol red thread test, mm | 21.40 ± 1.29 | 24.13 ± 1.60 | 0.49 |
| Fluorescein T-BUT, s | 3.82 ± 0.57 | 4.62 ± 0.86 | 0.38 |
| Corneal fluorescein staining, Oxford scale | 0.75 ± 0.16 | 0.33 ± 0.13 | 0.52 |
| Conjunctival Lissamine staining | 0.25 ± 0.09 | 0.63 ± 0.18 | 0.06 |
| Schirmer test, mm | 12.70 ± 1.85 | 14.50 ± 1.94 | 0.83 |

* Mann Whitney *U* test.

**Table 2.** Clinical Test Results

| Test | Exposure | SIC Mean ± SEM | SIC Range | SSC Mean ± SEM | SSC Range | *P* Value‖ |
|---|---|---|---|---|---|---|
| Modified SIDEQ | Pre | 9.61 ± 1.69 | 1.2–29.2 | 10.32 ± 3.40 | 1.5–43.1 | 0.65 |
| | Post | *12.38 ± 2.16*§ | 2.6–33.3 | 11.95 ± 3.47 | 2.1–46.2 | 0.63 |
| Tear osmolarity, mOsml/L | Pre | 325.70 ± 6.68 | 291–392 | 319.13 ± 4.75 | 297–347 | 0.58 |
| | Post | 328.20 ± 6.01 | 294–400 | 314.53 ± 4.00 | 289–340 | **0.04** |
| Phenol red thread test, mm | Pre | 20.95 ± 1.53 | 6.0–30.0 | 18.53 ± 1.51 | 6.0–30.0 | 0.58 |
| | Post | **16.75 ± 1.34**† | 4.0–29.0 | 20.00 ± 1.75 | 8.0–30.0 | 0.33 |
| Conjunctival bulbar hyperemia, mean | Pre | 1.10 ± 0.11 | 0.0–2.0 | 0.93 ± 0.14 | 0.0–2.0 | 0.80 |
| | Post | **1.43 ± 0.11**† | 0.5–2.5 | **1.33 ± 0.13**† | 1.0–2.5 | 0.44 |
| T–BUT, s | Pre | 2.18 ± 0.28 | 1.0–6.33 | 3.93 ± 0.87 | 1.0–13.0 | 0.18 |
| | Post | **1.53 ± 0.20**† | 0.66–4.33 | 4.51 ± 1.81 | 1.0–29.0 | **0.003** |
| Corneal fluorescein staining, Oxford scale | Pre | 0.50 ± 0.14 | 0.0–2.0 | 0.80 ± 0.24 | 0.0–2.0 | 0.68 |
| | Post | **1.25 ± 0.19**† | 0.0–3.0 | 1.00 ± 0.26 | 0.0–3.0 | 0.42 |
| Corneal intensity staining, Baylor scheme | | | | | | |
| Central | Pre | 0.25 ± 0.10 | 0.0–1.0 | 0.20 ± 0.14 | 0.0–2.0 | 0.63 |
| | Post | **0.70 ± 0.18*** | 0.0–2.0 | **0.67 ± 0.23*** | 0.0–2.0 | 0.63 |
| Nasal | Pre | 0.60 ± 0.15 | 0.0–2.0 | 1.00 ± 0.31 | 0.0–4.0 | 0.46 |
| | Post | **1.40 ± 0.23**† | 0.0–3.0 | 1.40 ± 0.32 | 0.0–4.0 | 0.91 |
| Temporal | Pre | 0.35 ± 0.15 | 0.0–2.0 | 0.73 ± 0.30 | 0.0–4.0 | 0.65 |
| | Post | **1.65 ± 0.24**‡ | 0.0–3.0 | 1.00 ± 0.32 | 0.0–4.0 | 0.08 |
| Superior | Pre | 0.05 ± 0.05 | 0.0–1.0 | 0.07 ± 0.07 | 0.0–1.0 | 0.80 |
| | Post | **0.25 ± 0.10*** | 0.0–1.0 | 0.27 ± 0.27 | 0.0–4.0 | 0.42 |
| Inferior | Pre | 1.15 ± 0.18 | 0.0–2.0 | 1.27 ± 0.33 | 0.0–4.0 | 0.61 |
| | Post | **2.25 ± 0.29**† | 0.0–4.0 | **2.00 ± 0.43*** | 0.0–4.0 | 0.61 |
| Corneal severity staining (inferior), Baylor scheme | Pre | 0.00 ± 0.00 | 0.0–0.0 | 0.13 ± 0.13 | 0.0–2.0 | 0.75 |
| | Post | **0.60 ± 0.20**† | 0.0–2.0 | 0.33 ± 0.19 | 0.0–2.0 | 0.46 |
| Conjunctival staining, mean | Pre | 0.60 ± 0.13 | 0.0–2.0 | 0.60 ± 0.16 | 0.0–2.0 | 0.85 |
| | Post | 0.68 ± 0.12 | 0.0–2.0 | 0.73 ± 0.18 | 0.0–2.5 | 0.96 |
| Schirmer test (without anesthesia), mm | Pre | 10.80 ± 1.87 | 2.0–32.0 | 14.33 ± 2.61 | 1.0–35.0 | 0.35 |
| | Post | 11.00 ± 1.95 | 1.0–34.0 | 13.20 ± 2.36 | 4.0–35.0 | 0.25 |

Comparison by Wilcoxon test before (pre) and after (post) 2 hour exposure to each environmental conditions (SIC and SSC) in an environmental chamber.

* $P \leq 0.05$.

† $P \leq 0.01$.

‡ $P \leq 0.001$.

§ $P = 0.06$.

‖ Comparison by Mann-Whitney *U* test between SIC and SSC.

Interestingly, DE patients exposed to SIC for 2 hours had significantly higher tear levels of IL-6 and MMP-9, whereas EGF tear levels decreased significantly (Fig. 1).

## Discussion

Using an environmental chamber, we exposed mild to moderate DE patients to an environmental condition (SIC) similar to that found in an aircraft cabin[6,7] for 2 hours. This caused an increase in symptoms and CFS, and a decrease in T-BUT and PRTT scores; additionally, there were variations in tear levels of some molecules, including a reduction in EGF and increases in IL-6 and MMP-9 (Fig. 2). On the contrary, the control condition (SSC) caused an increased central and inferior CFS, but no variation in the concentration of tear molecules.

The ocular surface is constantly exposed to both outdoor and indoor environments, and exposure to adverse conditions can impair the LFU.[22] High temperature,[23] low humidity,[22,23] air flow,[24] and high altitude (low atmospheric pressure)[25,26] are risk factors which can exacerbate DE symptoms. To our knowledge, there are no published studies reporting variations in clinical diagnostic tests, growth factors, and inflammatory tear molecules after DE patients are exposed to an adverse environment similar to that typically found in an aircraft cabin.

We used the OSDI instrument, which is frequently employed as an endpoint in ocular surface clinical trials, to classify individuals during recruitment based on its psychometrically tested validity and reliability to differentiate the severity of DE patients.[8] We specifically did not include severe DE patients whose LFU might already be highly altered. Rather, we recruited Level 1 and 2 DE patients already diagnosed and being treated in our eye clinic, having symptoms as measured with OSDI test.

To evaluate DE symptoms before and after each exposure, participants self-administered the modified-SIDEQ instrument. We used the same items of the original questionnaire, but we scored the results using a VAS scale that enlarged the scoring range from 0 to 10. Our aim with the SIDEQ modification was to apply an instrument more sensitive to small symptomatic changes.[27] Both DE groups showed a trend towards increased symptoms after both environmental conditions. However, we only found significant changes in burning and itching sensation in the DE group that underwent the SIC, as a result the modified SIDEQ global score also changed reaching almost statistical significance ($P = 0.06$). Our results are in accordance with those reported by Strøm-Tejsen et al.[28] who found that 50

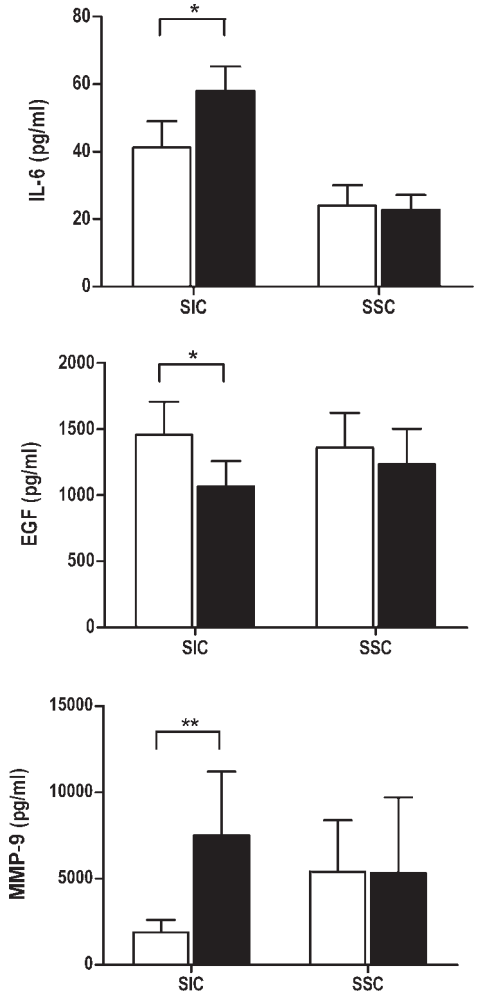


**Figure 1.** Tear molecules that showed significant changes. *Open bars*, pre-exposure level; *closed bars*, post 2 hour exposure level. Comparison by Wilcoxon test before and after exposure to environmental conditions: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$. SIC, simulated in-flight condition; SSC, Simulated standard condition.

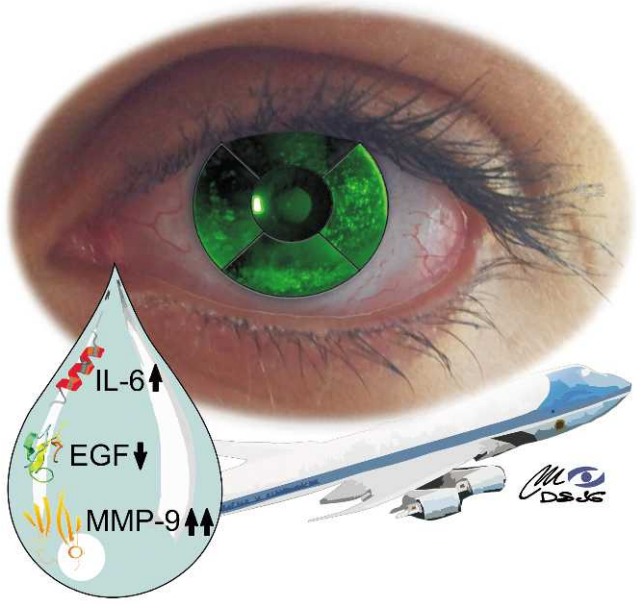


**Figure 2.** Clinical and molecular changes in dry eye patients after the simulated in-flight condition. This condition provoked an increase in conjunctival hyperemia and corneal staining (with the inferior area being most affected), and a decrease in tear quantity. Moreover, IL-6 and MMP-9 tear levels increased and EGF decreased. Airplane, IL-6, and EGF images adapted or reproduced from http://commons.wikimedia.org/wiki/File:Air_Force_One_over_Mt._Rushmore.jpg, http://en.wikipedia.org/wiki/File:IL6_Crystal_Structure.rsh.png, and http://en.wikipedia.org/wiki/File:1a3p_egf.png.

non-DE subjects subjectively perceived increasing eye dryness as RH decreased during a simulated 7 hour transatlantic flight using also a VAS scale. In our study, the increase in the reported symptoms under SIC could be related to the decreased tear film stability (as shown by the decreased T-BUT that these patients exhibited) provoked by the acceleration of the tear film thinning that occurs due to low humidity[22] and increased air flow.[24]

We performed the Schirmer test (without anesthesia) as it is one of the diagnostic criteria considered within the DE severity grading scheme proposed in the international DEWS 2007 report.[29] Prior to and after both environmental conditions, all participants achieved values well above 5 mm/5 minutes; thus, our mild DE patients did not have severe DE disease, as we intentionally meant. PRTT was more sensitive for the condition created in this study and actually decreased after SIC, probably due to the higher tear film evaporation under SIC compared with SSC. The PRTT has lower variability than the Schirmer test[30]; thus, it is likely that minor tear quantity changes can only be reliably detected by the PRTT as happened in our study. Both PRT and Schirmer test measure tear volume and/or production, but they are not interchangeable.[31] PRTT takes 15 seconds, Schirmer takes 5 minutes, a much longer time. Consequently, PRTT can measure the existing volume of tears in the meniscus and in the inferior conjunctival fornix whereas Schirmer test can also measure tear production in 5 minutes. PRTT hardly stimulates reflex tear, whereas Schirmer without topical anesthesia measures reflex tear production. Therefore, both tests are actually measuring different aspects,[14,32] are not interchangeable (a poor agreement has been previously described)[31] and can complement each other.

It has been reported that low RH values increase evaporative rates in both DE patients[33] and healthy subjects.[22] Likewise, higher RH increases T-BUT values, a reflection of improved tear film stability, presumably through decreased evaporation.[34] In contrast, reduced atmospheric pressure like that associated with high altitudes, increases evaporative water loss, as does increased air flow.[24] Therefore, the finding of reduced T-BUT values under SIC was expected; previous authors[22] reported this finding even in healthy patients under low RH (5%).

We found increased corneal global staining, which clinically reflects the degree of the epithelial injury[35] after SIC but not after SSC. The more detailed Baylor scheme showed some changes after SSC, specifically in central and inferior corneal regions, which were expected having in mind that these patients were performing near vision tasks. After SIC, CFS significantly increased in all areas, with the inferior cornea the most affected one; this could be due to the fact that these patients were looking slightly up (as when watching monitors in air cabins), and thus the area of cornea to be covered during blinking increases. It was actually reported that for control or DE patients, the average blink completes only about 70% of full eye closure for both near and distance vision, with the exposed 30% being located primarily in the inferior cornea.[36] Thus, it is not surprising that the inferior sector showed the highest epithelial damage in all of our participants performing cognitively demanding tasks. This is consistent with the

observation that T-BUT usually first occurs in the inferior cornea.[37]

Increased osmolarity has been described in patients having DE,[38] because in these patients, reduced tear secretion and/or increased evaporation results in loss of fluid that isotonic tears cannot overcome.[39] Our mild DE patients showed osmolarity values (Table 2) above the accepted DE diagnostic cutoff magnitude of 316 mOsm/L[12,13] before undergoing any condition. However, osmolarity did not show any change under SIC, where low RH and pressure could have provoked an increase. Perhaps these DE patients were not severe enough and they could still compensate that negative environment in their osmolarity values with increased aqueous tear flow. It is likely that tear osmolarity in more severe DE patients will increase, which remains to be proven.

We also wanted to explore the tear levels of some growth factors and inflammatory mediators that we,[40] and others,[18,41,42] have previously showed to be altered in DE syndrome, as well as some other molecules not yet reported. None of the studied molecules changed in the tears while exposed to SSC. In contrast, there were significant changes of some molecules during exposure to SIC. EGF is a growth factor that is secreted mainly by lacrimal glands and induces cellular proliferation and differentiation.[43] In our study, the concentration of EGF was significantly reduced in DE patients when subjected to the SIC, which agrees with previous studies by our group[40] and others,[18] where decreased EGF tear levels were associated with reduced corneal integrity.

IL-6 is a pro-inflammatory molecule frequently found in DE patients[18,40] and it has been suggested as a potentially useful biomarker for DE.[18] In our study, IL-6 significantly increased after SIC in agreement to previous results in DE patients.[18] MMP-9 promotes corneal extracellular matrix degradation and epithelial cell loss and is increased in tears of DE patients.[44] Our results also showed that MMP-9 tear concentrations were raised significantly in all DE patients exposed to SIC, changes consistent with those described by Chotikavanich et al.[45]

A limitation of the present study is that different DE patients were recruited for each environmental condition; however, we wanted to avoid any behavioral tendency in any group that could produce any bias in the study outcomes. Moreover, this study took 4 hours out of each patient's time (plus travelling time) and we thought recruitment could be compromised by asking them twice. We chose a rather low 45% RH level for the SSC because it is the most frequent RH in the geographic region where the recruited patients live (Valladolid and nearby cities),[46] being the average of nonarid regions.[33] However, this is not necessarily the optimum RH for the ocular surface and it is far from the RH found in coastal areas, which RH is usually 70% to 80%.[47] In fact, some DE patients had increased CFS and conjunctival hyperemia after being exposed to SSC. Finally, we cannot rule out that one test could have influenced the result of the next one, as the examination procedure was complex. We think that this influence does not affect the conclusions of this work for the following facts: we chose to perform the less invasive tests first, there was a 2 to 5 minute interval between tests and, more importantly, what we are reporting is the change between the pre- and post-exposure, so the same potential artifact would have influenced both examinations similarly.

In conclusion, we showed that an adverse environment similar to that found in an airplane with low humidity, low atmospheric pressure, and increased air flow produces an increase in clinical DE symptoms and signs. This condition also induced significant changes in tear molecule levels, EGF decreased and IL-6 and MMP-9 increased. Taking into account that this impairment occurred after only two hours of environmental stress in Level 1 and 2 symptomatic DE patients, and that a high percentage of flights last above 2 hours, DE therapeutic strategies will be increasingly needed due to the high prevalence of DE patients[4] that are part of the increasing population of airplane passengers worldwide.

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