Effect of Environmental Conditions on the Concentration of Tear Inflammatory Mediators During Contact Lens Wear

Vicente Martín-Montañez, PhD,† Amalia Enríquez-de-Salamanca, PhD,*† Alberto López-de la Rosa, MSc,* Alberto López-Miguel, PhD,* Itziar Fernández, PhD,*† Margarita Calonge, MD,*† José M. González-Méijome, PhD,‡ and María J. González-García, PhD*†*

Purpose: To analyze the influence of environmental conditions on the concentrations of tear inflammatory mediators during contact lens (CL) wear.

Methods: Fifty-four CL wearers completed 4 visits combining the bilateral use of omafilcon A or comfilcon A CL and a 90-minute exposure to 2 environmental conditions: standard [50% relative humidity (RH), 23°C, 930 mb] or adverse (5% RH, localized air flow, 23°C, 750 mb). Four microliters of tears was collected by capillarity from each subject. Changes in concentration of epidermal growth factor (EGF); interleukin (IL)-1 receptor antagonist, IL-1β, IL-2, IL-4, IL-6, and IL-8; tumor necrosis factor (TNF) α; monocyte chemoattractant protein-1; and matrix metalloproteinase (MMP)-9 were analyzed. The effects of the environment, CL type, and symptoms were evaluated using a 3-way mixed analysis of variance with repeated measures.

Results: Under the standard condition, EGF significantly increased [0.36; 95% confidence interval (CI), 0.08 to 0.64], and IL-1β (−0.48; 95% CI, −0.84 to −0.12) and IL-2 (−0.48; 95% CI, −0.87 to −0.09) significantly decreased. Under the adverse condition, IL-6 significantly increased (0.35; 95% CI, 0.09 to 0.62). Comparing conditions, EGF change was significantly lower ($P = 0.02$) and IL-1β, IL-2, IL-6, and TNF-α changes were significantly higher ($P \leq 0.04$) under the adverse condition. Additionally, IL-1β significantly decreased with comfilcon A (−0.51; 95% CI, −0.88 to −0.15), being significantly lower ($P = 0.01$) than the change with omafilcon A (0.06; 95% CI, −0.23 to 0.35).

Conclusions: The secretion of several tear inflammatory mediators during CL wear differs depending on the environmental conditions and the CL type used. These outcomes might help to understand the effect of the environment and CL materials on the ocular surface of CL wearers.

Key Words: cytokines, chemokines, inflammation, environment, contact lens

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Contact lens (CL) wearers are daily exposed to artificially controlled environments such as air-conditioned offices, buildings, or aircraft cabins. These indoor spaces involve challenging environmental conditions such as low relative humidity (RH), air flow, and/or decreased barometric pressure. Airplane cabins in particular, because of their characteristics regarding RH, air renewal, etc., represent an adverse environment, which combines these conditions all together.[1] Previous studies have shown that adverse conditions elicit the development of dryness symptoms in dry eye patients and CL wearers.[2–8] These desiccating scenarios also have a negative impact on clinical signs of the ocular surface,[2,9–11] including a decrease in tear volume and stability and an increase in conjunctival hyperemia and corneal staining. As the presence of a CL on the ocular surface reduces tear film stability,[12] CL wear in adverse environments is likely to exacerbate further these ocular symptoms.

It is known that some CL-related complications are somewhat mediated through the release of tear inflammatory mediators, such as giant papillary conjunctivitis, which presents altered tear levels of eotaxin,[13] or corneal neovascularization, which is mediated by vascular endothelial growth factor.[14] Likewise, the concentration of several tear molecules, such as interleukin (IL)-6, IL-8, and epidermal growth factor (EGF),[15–18] has been shown to be upregulated in CL wearers. Moreover, CL type could be key in this, as differences have been found between hard and soft CL wearers.[16,19] These findings suggest that there may be an underlying inflammatory process related to CL wear. The expression of some tear proteins has also been found altered in subjects with CL-related discomfort[20]; this discomfort can lead to decreased wearing time and discontinuation of CL wear.[21]

Exposure to desiccating environments exacerbates dry eye clinical signs[2,9,10] and increases certain proinflammatory molecules regardless of the presence of dry eye disease.[10] We hypothesized that transient exposure to an artificially adverse environment could also elicit the expression of some

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From the *IOBA, University of Valladolid, Valladolid, Spain; †Networking Research Center on Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN), Valladolid, Spain; and ‡Clinical and Experimental Optometry Research Laboratory, Center of Physics, University of Minho, Braga, Portugal.
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Reprints: María J. González-García, PhD, IOBA, University of Valladolid, Paseo de Belén, 17, 47011 Valladolid, Spain (e-mail: mjgonzalez@ioba.med.uva.es).

proinflammatory mediators in CL wearers. With that in mind, the purpose of the study was to analyze the influence of controlled environmental conditions, CL type, and CL-related symptoms on the concentration of tear inflammatory mediators in CL wearers.

MATERIALS AND METHODS

Ethical Issues

The study complied with the tenets of the Declaration of Helsinki, and the protocol was approved by the University of Valladolid Ethics Committee. The nature of the research and protocols was explained to the subjects before written informed consent was obtained during the preliminary visit.

Participants

Fifty-four soft CL wearers [20 men and 34 women; mean age ± standard deviation (SD), 20.0 ± 7.3 years; range, 18–45] were included. The criteria for selecting subjects were age between 18 and 45 years, a myopic spherical equivalent between −1.00 and −5.00 diopters (D), astigmatism error less than or equal to 0.75 D, and having worn CLs for at least the last 6 months before the study. Exclusion criteria were taking systemic or ocular medication (different from artificial tears), ocular abnormalities, and a history of ophthalmic disease or surgery, including refractive procedures.

The ocular surface disease index (OSDI) questionnaire was administered to categorize subjects based on their discomfort with their habitual CL wear (discomfort-based grouping). Those having an OSDI score <15 were classified as asymptomatic, and those having a score ≥ 15 were classified as symptomatic.[22]

At the preliminary visit (V0), all subjects were fitted with the 2 study CLs—omafilcon A and comfilcon A (CooperVision, Irvine, CA)—to confirm that both types of CLs fit each subject properly. These CLs were chosen because of their similarities in CL parameters and physical properties of their CL blister solutions (osmolarity, pH, surface tension, and viscosity).[23]

The right eye was chosen as the study eye for tear molecule analysis, despite the fact that the CLs were worn bilaterally.

Study Protocol

The recruited subjects were exposed to 2 different environmental conditions wearing each of the 2 CL types for each exposure, making a total of 4 visits by each subject, thus volunteers wore each CL type under 2 different environmental conditions. At the beginning of each visit, subjects were bilaterally fitted with the randomized CL, wore the CLs for 15 minutes, and immediately after performing the tear collection, they were exposed to the randomized environmental condition. The order in which participants were exposed with each CL under each environmental condition was randomized, and visits were spaced between 2 and 5 days. Subjects were instructed not to use their CLs or artificial tears from 24 hours before starting the study until after they had finished all visits.

Environmental Conditions

Subjects were exposed within an environmental chamber (CERLab, IOBA, University of Valladolid)[9] to 2 different controlled environmental conditions: (1) "standard condition" (50% RH, 23°C temperature, and 930 mb of atmospheric pressure, which is the average pressure in Valladolid, Spain) and (2) "adverse condition" (5% RH, localized air flow—mean air velocity 0.43 m/s, 23°C temperature, and 750 mb atmospheric pressure—similar to that typically found within an airplane cabin during flight).[24–26] Environmental chamber exposure lasted 90 minutes, while the individuals were seated watching a film on a 55-inch television (LG Electronics Inc, Gumi, South Korea).

Tear Sample Collection

Tear sample collection was performed twice per visit: (1) after wearing the CL for 15 minutes and just before the exposure to each environmental condition (PRE) and (2) immediately after 90 minutes of exposure (POST).

A 4-μL sample of basal tears was collected from the external canthus using a glass capillary tube (Drummond Scientific, Broomall, PA) in a nontraumatic way, trying to avoid reflex tear secretion as much as possible. Tear samples were diluted (1/10) in assay buffer and frozen as described previously.[27]

Analysis of Tear Molecule Concentration

The concentrations of EGF; IL-1 receptor antagonist (RA); IL-1β, IL-2, IL-4, IL-6, IL-8; tumor necrosis factor (TNF) α; monocyte chemoattractant protein-1 (MCP-1); and matrix metalloproteinase-9 (MMP-9) were measured simultaneously with a 10-plex immunobead-based assay (10X-plex magnetic human cytokine/MMP-9 panel; Millipore, Billerica, MA) in a Luminex IS-100 instrument (Luminex Corp, Austin, TX). The samples were analyzed according to the manufacturer's procedure as previously described.[27] The minimum detectable concentrations (in picograms per milliliter) for molecules analyzed were as follows: 1.23 for EGF, IL-1RA, IL-4, IL-8, MCP-1, and MMP-9; 1.18 for TNF-α; 1.16 for IL-2; 1.12 for IL-6; and 1.05 for IL-1β.

In some cases, the assayed molecule was undetectable. Cytokine levels below the limit of detection were imputed using the robust regression on order statistics method introduced by Helsel[28] and implemented in the NADA (nondetects and data analysis) package.[29] However, molecules detected in less than 50% of the samples in every condition were not analyzed further because the statistical analysis might be biased.[30]

Data Analysis

The sample size was calculated with the online freeware "Power Analysis for ANOVA Designs".[31] It took into account that 2 groups and 4 clinical scenarios were compared, level of significance was determined as 0.05, effect size as 0.40,[32] and statistical power as 80%. Based on these parameters, sample size calculated was 25 subjects for each symptomatic and asymptomatic group. We included 2 additional subjects per

group in case of dropout; therefore, the total sample size was 27 subjects for each group.

Sample distributions for quantitative variables were analyzed using the Mann–Whitney *U* test, whereas qualitative variables were compared using either the χ^2 test or the Fisher exact test if cell sizes were too small.

Tear molecule analysis data were log-transformed (log 2), which normalizes the tear concentration data, before conducting statistical analysis. The change in tear molecule concentration between both moments of environment exposure (PRE and POST) was calculated as the difference with the following formula: change = $\log_2(Y_{post}) - \log_2(Y_{pre})$, where "$Y_{post}$" is the "Y" tear molecule concentration after the exposure and "Y_{pre}" is the "Y" tear molecule concentration before the exposure. The main advantage of this formula is that equal positive and negative change values will be symmetric and reciprocal, that is, a change of −1 means that the amount of the molecule is half of the previous value and a change of +1 means double. The "change" numerical value thus obtained was considered as the main variable. Changes in molecules whose 95% confidence interval (CI) did not include the "0" value were considered statistically significant.

A multivariable analysis, a repeated-measures analysis of variance (ANOVA), was used to analyze the effect of environmental condition (standard and adverse), CL type (omafilcon A and comfilcon A), and discomfort-based grouping (asymptomatic and symptomatic subjects), and their interactions, on the change in tear molecule concentration. Two intrasubject (environmental condition and CL type) and 1 intersubject (discomfort-based grouping) factors, and their interactions, were considered. Pairwise comparisons were based on Student *t* tests. *P*-values were adjusted for multiple testing by the Holm method.[33]

Statistical analysis was carried out by a licensed statistician (coauthor I. Fernández) using R Statistical Software.[34] Package Car[35] was used to fit repeated-measures ANOVA models. *P* values less than or equal to 0.05 were considered statistically significant.

RESULTS

The OSDI-based classification divided the participants into an asymptomatic group (11 males and 16 females; white; mean age 25.8 ± 6.0 years) and a symptomatic group (9 males and 18 females; white; mean age 28.8 ± 8.3 years). None of the participants suffered from systemic diseases. Clinical variables included at the preliminary visit for both groups are shown in Table 1. The subject sex and age did not differ significantly ($P = 0.573$ and $P = 0.215$, respectively) between the groups. Likewise, no significant differences were found between the groups in terms of CL wear time ($P = 0.476$), 7.1 ± 5.3 years for the asymptomatic group and 6.7 ± 4.5 years for the symptomatic one. CL wear schedule did not differ between groups either ($P = 0.538$). In the asymptomatic group, there were 3, 2, and 22 volunteers whose CL schedule was daily, biweekly, and monthly, respectively. In the symptomatic group, there were 6, 3, and 18 volunteers following the same CL schedules, respectively. In contrast, we found significant differences ($P = 0.01$) between groups for artificial tear use before the study; no participant from the asymptomatic group used them, whereas 7 out of 27 symptomatic wearers did.

TABLE 1. Clinical Variables of the Groups Included at the Preliminary Visit

Variables	Asymptomatic Group: Mean ± SD or Median (Interquartile Range)	Symptomatic Group: Mean ± SD or Median (Interquartile Range)	*P*
Visual acuity (logarithm of the minimum angle of resolution)	−0.06 ± 0.05	−0.05 ± 0.05	0.45
BUT (s)	6.5 ± 3.5	4.6 ± 3.1	0.03*
Corneal staining (Oxford scale)	0 (0–0)	0 (0–0)	0.77
Schirmer I test (mm)	24.6 ± 10.6	21.0 ± 11.5	0.28

*P-value < 0.05.

The mean percentage of detection of each molecule (including visits 1–4, before and after environmental exposure) was as follows: EGF (95%), IL-1RA (94%), IL-1β (50%), IL-2 (56%), IL-4 (49%), IL-6 (71%), IL-8 (95%), TNF-α (53%), MCP-1 (96%), and MMP-9 (78%). Each molecule concentration obtained during each visit is detailed in Supplemental Digital Content 1 (see Table, http://links.lww.com/ICO/A436).

Effect of the "Environment"

Analyzing the changes provoked by each condition, the standard condition exposure provoked a significant increase in the concentration of EGF (0.36; 95% CI, 0.08 to 0.64) and a decrease of IL-1β (−0.48; 95% CI, −0.84 to −0.12) and IL-2 (−0.48; 95% CI, −0.87 to −0.09). A significant increase of IL-6 level (0.35; 95% CI, 0.09 to 0.62) was observed after the adverse condition exposure (Fig. 1). Comparing adverse and standard conditions, the adverse condition provoked significantly lower change than the standard condition for EGF ($P = 0.02$) and significantly higher for IL-1β ($P = 0.029$), IL-2 ($P = 0.016$), IL-6 ($P = 0.045$), and TNF-α ($P = 0.029$) (Fig. 1).

The other molecules analyzed (IL-1RA, IL-4, IL-8, MCP-1, and MMP-9) were unaffected by the environment ($P > 0.05$).

Effect of the "CL Type"

Tear levels of IL-1β significantly decreased when wearing the comfilcon A CL (−0.51; 95% CI, −0.88 to −0.15), whereas no significant change was reported with omafilcon A (0.06; 95% CI, −0.23 to 0.35) (Fig. 2). Moreover, the change of IL-1β was significantly different comparing both CLs ($P = 0.014$) (Fig. 2).

The CL type did not show any effect on the other tear molecules ($P > 0.05$): EGF, IL-1RA, IL-2, IL-4, IL-6, IL-8, TNF-α, MCP-1, and MMP-9.

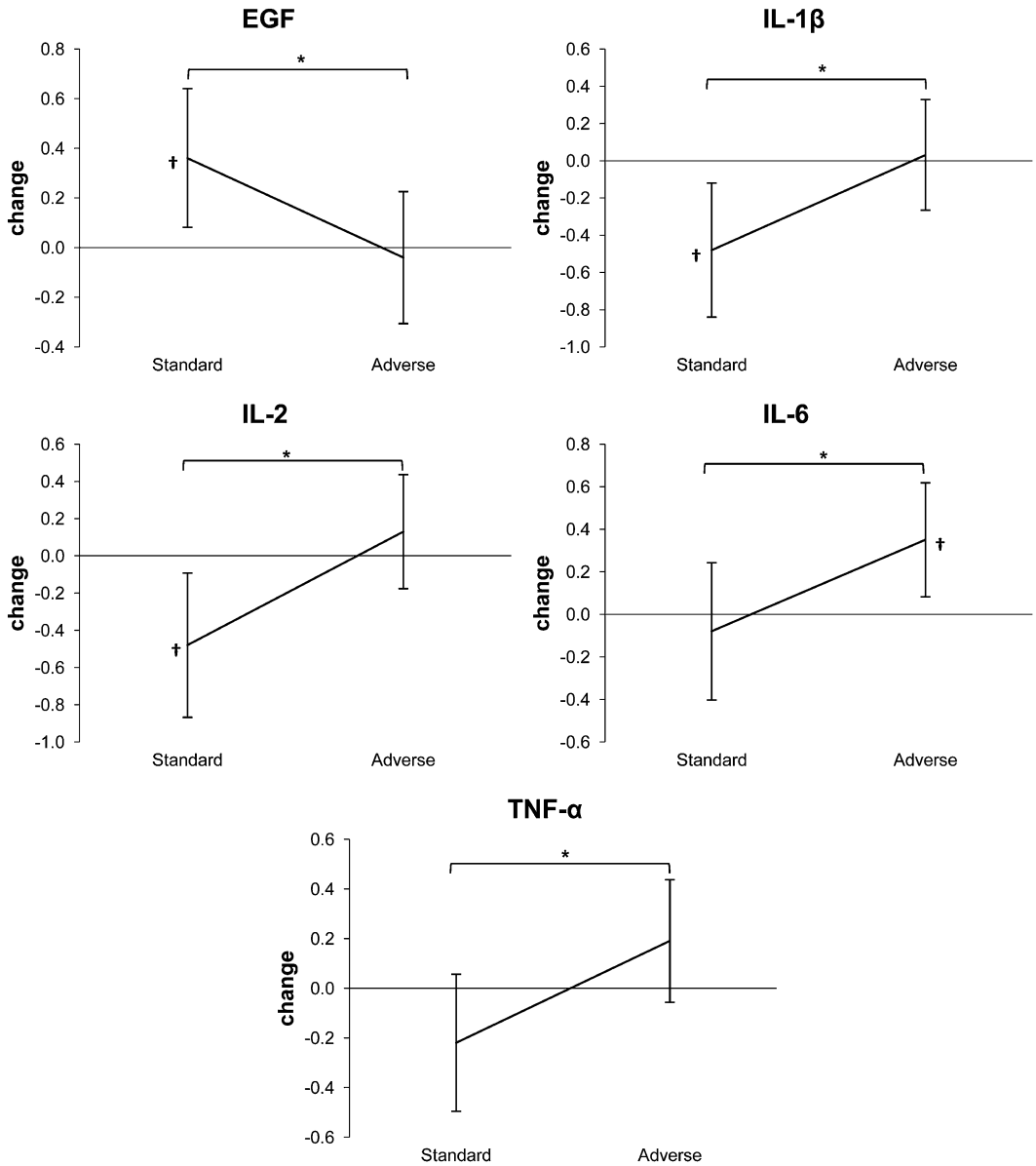

FIGURE 1. Environmentally produced changes in EGF, IL-1β, IL-2, IL-6, and TNF-α concentrations. *Significant ($P < 0.05$) difference between the changes produced by the 2 environments. †Significant ($P < 0.05$) environmentally produced change in molecule concentration.

Effect of the "Symptom-Based Grouping"

No significant effects of the symptom-based grouping were found in any tear molecule analyzed ($P > 0.05$): EGF, IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-8, TNF-α, MCP-1, and MMP-9.

Effect of the Interaction "Environment and CL Type"

Under the standard condition, there was a significant decrease in IL-1β concentration when wearing comfilcon A CL (−1.05; 95% CI, −1.56 to −0.54), but no change was observed with omafilcon A (0.08; 95% CI, −0.40 to 0.57). These changes in IL-1β concentration were significantly different ($P = 0.001$) comparing both CLs (Fig. 3). No effects were found for this interaction under the adverse condition (Fig. 3).

No significant results were found for the remaining molecules ($P > 0.05$): EGF, IL-1RA, IL-2, IL-4, IL-6, IL-8, TNF-α, MCP-1, and MMP-9, although the change in MMP-9 showed a borderline significant effect ($P = 0.055$).

Effect of the Interaction "Environment and Symptom-Based Grouping"

No significant effects were found for this interaction in any tear molecule analyzed ($P > 0.05$): EGF, IL-1β, IL-1RA, IL-2, IL-4, IL-6, IL-8, TNF-α, MCP-1, and MMP-9.

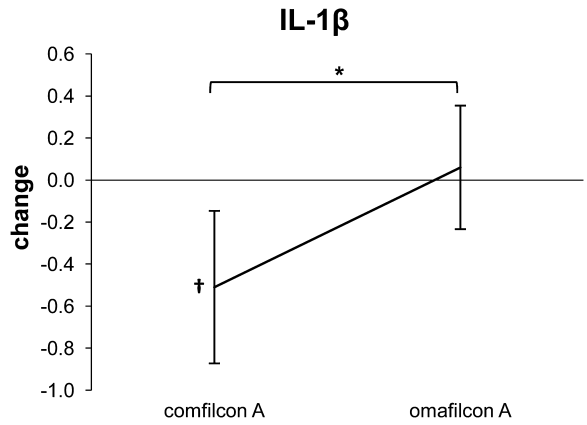

FIGURE 2. Changes produced in IL-1β concentration by CL type. *Significant ($P < 0.05$) difference between the changes produced by the 2 lenses. †Significant ($P < 0.05$) change produced by comfilcon A in molecule concentration.

Effect of the Interaction "CL Type and Symptom-Based Grouping"

A significant decrease was found for asymptomatic subjects with comfilcon A for IL-1β (–0.90; 95% CI, –1.40 to −0.40), but not with omafilcon A. A significant increase was found with omafilcon A for IL-6 (0.50; 95% CI, 0.17 to 0.83) and IL-8 (0.46; 95% CI, 0.07 to 0.86), but not with comfilcon A (Fig. 4). Moreover, changes found for both CLs were significantly different for IL-1β ($P = 0.001$), IL-6 ($P = 0.040$), and IL-8 ($P = 0.016$). No effects were found for this interaction in symptomatic subjects (Fig. 4).

The other tear molecules (EGF, IL-1RA, IL-2, IL-4, IL-6, IL-8, TNF-α, MCP-1, and MMP-9) were not affected by this interaction ($P > 0.05$).

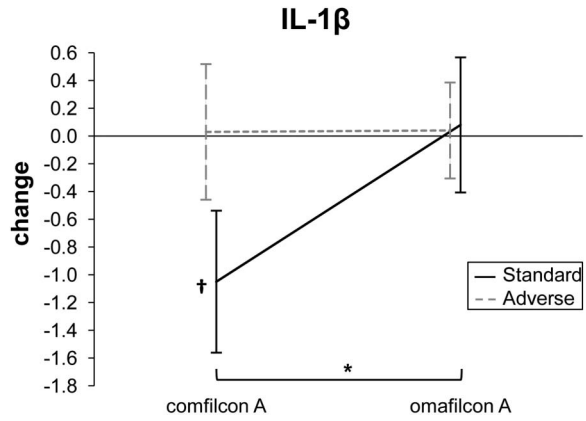

FIGURE 3. Changes in IL-1β concentration produced by the interaction between the environment and CL type. *Significant ($P < 0.05$) difference between the changes produced by the 2 lenses under the standard condition. †Significant ($P < 0.05$) change in molecule concentration produced by comfilcon A under the standard condition.

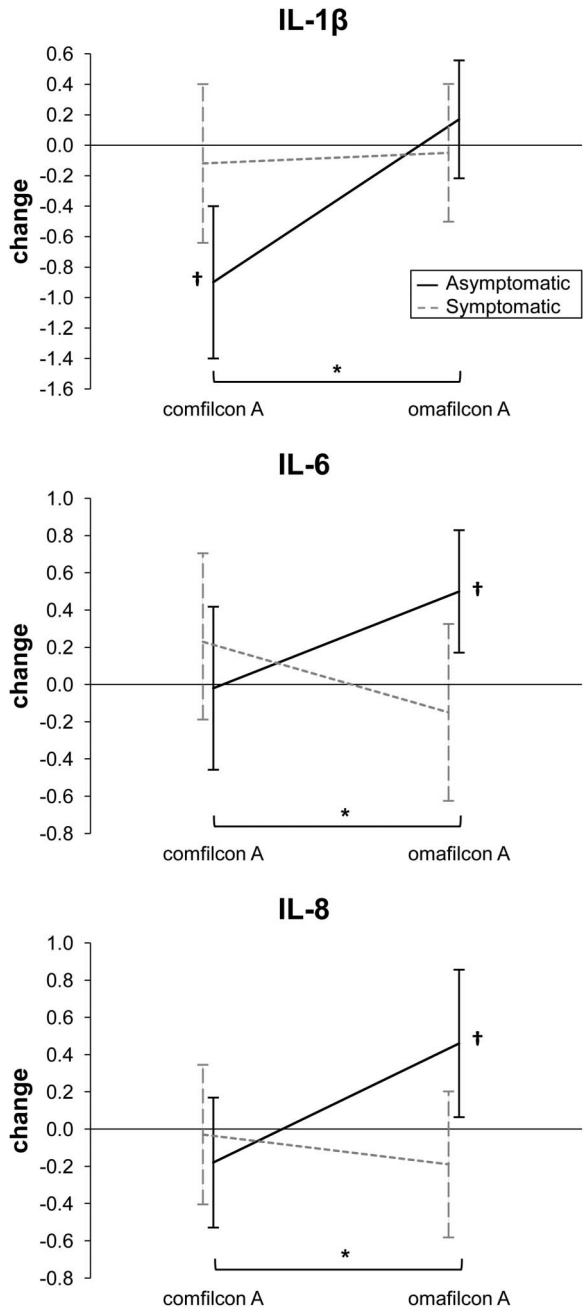

FIGURE 4. Changes in IL-1β, IL-6, and IL-8 concentrations produced by the interaction between CL type and symptom-based grouping. *Significant ($P < 0.05$) difference between the changes produced by the 2 lenses in the asymptomatic group. †Significant ($P < 0.05$) change produced by the CL type in the molecule concentration.

DISCUSSION

In this study we have found that, after 2 hours of CL wear, an environmentally adverse condition provoked a more inflammatory profile (lower EGF and higher IL-1β, IL-2, IL-6, and TNF-α concentrations) than a standard condition in CL wearers. Surprisingly, it is the standard condition that mainly affected the molecule levels, provoking an increase of

EGF and decrease of IL-1β and IL-2, whereas the adverse condition only provoked an increase of IL-6. In addition, there were also differences between CLs evaluated: patients wearing comfilcon A showed a decrease of IL-1β concentration, but this reduction was not seen with omafilcon A.

Our outcomes showed that the standard condition caused an effect that could be considered "protective" because EGF tear levels significantly increased but the concentration of inflammatory cytokines such as IL-1β and IL-2 significantly decreased. In contrast, this effect was not observed after our volunteers were exposed to the adverse condition. Instead, the changes in molecule concentrations constituted a "proinflammatory" scenario because IL-6 tear levels significantly increased. Besides, changes observed in EGF, IL-1β, IL-2, IL-6, and TNF-α concentrations were significantly different from those observed after the standard condition, which further shows the "proinflammatory" effect of this desiccating condition simulating an air flight. All these results are in agreement with previous outcomes from our group where the effect of the environmental condition was studied in both healthy and dry eye patients.[9,10] These studies have shown that under an adverse condition (simulating an aircraft cabin), EGF concentration was significantly reduced and IL-6 tear levels were significantly increased in both healthy[10] and dry eye patients,[9] an effect that was not found under a standard condition either.[9] Although we found no significant decrease in EGF concentration under the adverse condition, these levels were significantly lower than those found under the standard condition. These similarities between these studies[9,10] and the findings in our CL users further confirm the potential of desiccating indoor environments to stimulate an inflammatory response not only in healthy subjects and dry eye patients but also in CL wearers.

Our results showed that the IL-1β tear concentration was significantly reduced after wearing the comfilcon A in comparison with the omafilcon A, and also when wearing the comfilcon A under the standard condition in comparison with the other 3 situations. Despite the fact that no studies have yet detected IL-1β altered in CL wearers, it has been found to be increased in tears in dry eye patients, and even shows a positive correlation with dry eye syndrome severity.[36] Thus, the decrease of IL-1β found might indicate that the use of comfilcon A under a standard condition may provide a less proinflammatory environment than the other situations. This result might be related to the higher oxygen permeability of comfilcom A (a silicone hydrogel CL), which in combination with a standard condition, produces a less proinflammatory effect on the ocular surface.

Similarly, the outcomes of the interaction between CL type and symptom-based grouping revealed that the IL-1β, IL-6, and IL-8 tear concentrations were dependent on the CL type but only for asymptomatic subjects. IL-1β has been found elevated in tears of dry eye patients,[36] IL-6 has been shown to be a mediator of pain,[37] and IL-8 can induce hyperalgesia[38]; thus, changes in their concentrations might be related to CL discomfort. Therefore, our results may indicate that in symptomatic subjects the effect of the CL material would be minor because the ocular surface is already altered, whereas in asymptomatic wearers, CLs can produce different inflammatory levels, depending on the CL type used.

This study has some limitations. First, only 2 CL types were tested; other commercially available CL types could behave differently on the eye. Therefore, the results obtained are largely related to the CLs themselves and should be applied with caution to other CL designs and materials. Second, tear collection was always performed when the subjects were wearing CLs; consequently, our results describe the effect that the environmental conditions have on the ocular surface during CL wear. Therefore, as no tear collection was done without CL wear, the effect that these environmental conditions have on these subjects in the absence of CL wear was not studied and is unknown. And finally, CL wear has been found to decrease the tear film break-up time[39] and the tear meniscus volume[40] and to increase tear osmolarity.[41] These findings might be associated with a reduction in the aqueous component of tears and could have an impact on the concentration of tear molecules (eg, cytokine levels) because of the change in the dilution rate, affecting our results. Further studies in CL wearers about the effect of environmental conditions including some other CL types and tear collection at different times and in the absence of CL wear are warranted.

In conclusion, the secretion of several tear inflammatory mediators during CL wear differs depending on the environmental conditions, standard or adverse, and the CL type, omafilcon A or comfilcon A. This might help to understand the effect of the environment and CL materials on the ocular surface of CL wearers. Differences observed between symptomatic and nonsymptomatic CL wearers will also provide insights for future strategies to overcome CL discomfort and inflammatory events in CL wearers.

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