# Changes in Diadenosine Polyphosphates during Alignment-Fit and Orthokeratology Rigid Gas Permeable Lens Wear

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**PURPOSE.** To evaluate the levels of dinucleotides diadenosine tetraphosphate ($Ap_4A$) and diadenosine pentaphosphate ($Ap_5A$) in tears of patients wearing rigid gas permeable (RGP) contact lenses on a daily wear basis and of patients wearing reverse-geometry RGP lenses overnight for orthokeratology treatment.

**METHODS.** Twenty-two young volunteers (10 females, 12 males; $23.47 \pm 4.49$ years) were fitted with an alignment-fit RGP lens (paflufocon B) for a month, and after a 15-day washout period they were fitted with reverse-geometry RGP lenses for corneal reshaping (paflufocon D) for another month. During each period, tears were collected at baseline day 1, 7, 15, and 28. $Ap_4A$ and $Ap_5A$ were measured by high-pressure liquid chromatography (HPLC). Additionally, corneal staining, break-up time (BUT), Schirmer test, and dryness symptoms were evaluated.

**RESULTS.** $Ap_4A$ concentrations increased significantly from baseline during the whole period of daily wear of RGP lenses ($P < 0.001$); concentration was also significantly higher than in the orthokeratology group, which remained at baseline levels during the study period except at day 1 ($P < 0.001$) and day 28 ($P = 0.041$). While BUT and Schirmer remained unchanged in both groups, discomfort and dryness were significantly increased during alignment-fit RGP daily wear but not during the orthokeratology period.

**CONCLUSIONS.** Daily wear of RGP lenses increased the levels of $Ap_4A$ due to mechanical stimulation by blinking of the corneal epithelium, and this is associated with discomfort. Also, orthokeratology did not produce symptoms or signs of ocular dryness, which could be a potential advantage over soft contact lenses in terms of contact lens-induced dryness. (*Invest Ophthalmol Vis Sci.* 2012;53:4426–4432) DOI: 10.1167/iovs.11-9342

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Wearing contact lenses (CL) is one factor that can disturb tear dynamics, potentially resulting in ocular discomfort, dryness, and ocular surface disease.[1–4] CL-related comfort issues might involve a wide range of factors, which are particularly active under certain environmental conditions.[5] More than half of CL wearers self-report dry eye symptoms.[1] Some of these wearers reduce their lens wear time and some eventually abandon lens wear. Over 15% of lens wear dropout is attributed to dryness, and nearly 30% to ocular discomfort.[6,7]

Regarding rigid gas permeable lenses (RGP), it is commonly accepted that initial comfort will be low, increasing during the first few days until regular use during the whole day.[8] A unique case within the RGP field is overnight orthokeratology for corneal reshaping or corneal refractive therapy (CRT). Clinicians are aware that the eyelids play a major role in RGP, CL-related discomfort during blinking, and this is common at first and within the adaptation period of all RGP lenses. This inconvenience is minimized in CRT[9] because the lenses are worn overnight with no blinking interactions.

The Dry Eye Workshop proposed to diagnose dry eye by combining different tests to identify signs and symptoms.[10] However, it is necessary to be aware that the diagnosis of dry eye is difficult due to the poor correlation between dry eye signs and symptoms.[11] Analytical techniques are providing valuable information about new components in tears that may have important biochemical and physiological functions.[12] Among these tear components, diadenosine polyphosphates emerge as interesting compounds. They are released in the tear film by corneal shear stress during blinking, inducing an increase of dinucleotide concentration,[13] and have important intracellular and extracellular physiological actions. It is known that $Ap_4A$ and $Ap_5A$ concentrations increase in patients with dry eye symptoms and Sjögren syndrome, suggesting the possibility of these compounds being used as objective markers to diagnose and classify dry eye.[13,14] Moreover, a single-dose topical application of the dinucleotide $Ap_4A$ in rabbits has demonstrated to stimulate tear secretion, which confers to these compounds a secretagogue action.[15] $Ap_4A$ also has the ability to accelerate corneal wound healing in New Zealand white rabbits.[16]

Due to the involvement of diadenosine polyphosphates in the ocular surface physiopathology and its release mechanism related with blinking, it is a matter of interest to investigate a possible relationship between the concentrations of these substances and the development of dry eye in CL wearers—in this case, RGP lenses with different wearing schedules. With the aim of examining this relationship, the present work described variations in the levels of diadenosine polyphosphates ($Ap_4A$ and $Ap_5A$) during 1 month with alignment-fit aspheric RGP lenses worn on a daily basis, followed by a washout period of 2 weeks, and finally another month with reverse geometry for CRT worn overnight. Furthermore, study authors examined tear volume and stability, corneal staining,

**TABLE 1.** Demographic Information of the Population Enrolled in the Study

| Parameter | Values |
| --- | --- |
| Number of patients | 22 |
| Mean age (years $\pm$ SD) | 23.47 $\pm$ 4.49 |
| Age range (years) | 19, 36 |
| Sex (male/female) | 12, 10 |
| Mean corneal astigmatism (D $\pm$ SD) | −0.83 $\pm$ 0.36 |
| Range of corneal astigmatism (D) | −0.10, −1.14 |
| Mean flat keratometry (D) | 42.91 $\pm$ 1.13 |
| Mean steep keratometry (D) | 43.74 $\pm$ 1.36 |
| Mean refractive sphere (D $\pm$ SD) | −1.92 $\pm$ 1.23 |
| Range of refractive sphere (D) | −0.25, −5.25 |
| Mean refractive cylinder (D $\pm$ SD) | −0.30 $\pm$ 0.33 |
| Range of refractive cylinder (D) | 0, −0.75 |

All subjects underwent both parts of the study either with the alignment-fit RGP lens on a daily wear basis or with the reverse-geometry lens worn overnight.

and discomfort and dryness symptoms, which may be present during dry eye.

## METHODS

Twenty-two patients (12 males and 10 females) with ages ranging from 19 to 36 years (average 23.47 $\pm$ 4.49 years) were recruited from the student population at the Clinical & Experimental Optometry Research Lab (University of Minho, Braga, Portugal). The study was conducted in compliance with good clinical practice guidelines, informed consent regulations, and the tenets of the Declaration of Helsinki. All of the subjects enrolled in the study were adults older than 18 years who were able to give informed consent. More detailed demographic characteristics of the population are shown in Table 1. After the purpose of the study was explained and all doubts clarified to the participants, a consent form was presented and signed by both the patient and the researcher.

Fitted lenses were made of high oxygen permeability materials (paflufocon D for CRT and paflufocon B for RGP lenses). Technical parameters of the lenses are shown in Table 2.

Patients underwent a complete preliminary optometric examination including objective and subjective refraction, using the maximum plus for better visual acuity as endpoint criterion. Ocular surface inspection included slit-lamp examination and keratometry. The lenses

were fitted in conformity with the manufacturers' recommendations. When lenses were prescribed, instructions about wearing schedule, insertion, removal, and care recommendations were given to the patient. The care system consisted of a multipurpose solution for RGP lenses (Boston Simplus; Bausch & Lomb, Rochester, NY). During orthokeratology lens wear, patients were instructed to apply rewetting drops (Moisture Eyes; Bausch & Lomb) at insertion in the evening and before removal of their lenses in the morning.

## Trials

Aftercare visits were scheduled at days 1, 7, 15, and 28. Tear analysis, corneal staining, and recording of subjective symptoms including dryness and discomfort using the Dry Eye Questionnaire (DEQ) were carried out during these visits.[17,18]

Tear secretion was measured using the Schirmer I test (without anesthesia). Tear collection was always performed following Van Bijsterveld criteria.[19] The Schirmer strip (Tear Flo; HUB Pharmaceuticals, Rancho Cucamonga, CA) was placed on the temporal tarsal conjunctiva of the lower lid for 5 minutes with the eyes closed. The volume of tears was recorded as millimeters of moistened strip, and the wetted ends of the Schirmer strip were placed in Eppendorf tubes containing 500 μL of ultrapure water. The samples were then frozen until the high-pressure liquid chromatography (HPLC) analysis was performed.[13] Tear collection during RGP daily wear regimen was performed in the evening after using lenses with 15 minutes of washout. In the case of orthokeratology, the Schirmer test was performed in the morning after removing the lenses and with the same time of washout.

After the Schirmer I test, fluorescein was applied to evaluate BUT and corneal staining. In order to warrant repeatability of the staining procedure, a solution was prepared using a 10% concentration of sodium fluorescein diluted in saline (NaCl 0.9%). For each application, a micropipette with 5 μL of diluted fluorescein solution was applied in the inferior conjunctival sac and 20 seconds later, BUT was analyzed using a chronograph to record the tear film break-up time after the patient was asked to blink twice and remain with the eyes open. The cornea was divided in five areas to record the grading staining as proposed by the Report of the National Eye Institute and Industry-Sponsored Dry Eye Workshop,[20] using the Cornea and Contact Lens Research Unit grading scales.[21]

The patients were asked to characterize the frequency of two main symptoms of dry eye—namely, discomfort and dryness—which appear on the DEQ, elaborated by Begley et al.[17] DEQ was designed to assess the prevalence, frequency, and diurnal severity of ocular surface symptoms such as discomfort, dryness, visual changes, soreness,

**TABLE 2.** Technical Parameters of the Lenses Used in the Study

| Parameter | Alignment-Fit Aspheric Lens | Reverse-Geometry Lens |
| --- | --- | --- |
| Manufacturer | Lenticon (Madrid, Spain) | Paragon Vision Science (Mesa, AZ) |
| Material (USAN) | Paflufocon B | Paflufocon D |
| Brand | Oxicon HDS | Paragon CRT |
| Manufacturing process | Lathe-cut | Lathe-cut |
| Back surface geometry | Central 5 mm: spherical | Sigmoid geometry |
| | Periphery: aspheric (ecc. = 0.35) | |
| Front surface geometry | Spheric | Mirrored with anterior surface |
| Overall diameter (mm) | 9.80 | 10.50 |
| Optic zone diameter (mm) | 9.00 | 6.00 |
| Center thickness (@−3.00 D in mm) | 0.180 | 0.166 |
| Dk (barrer) | 58 | 100 |
| Contact angle | 14.8 | |
| Hardness (shore) | 84 | |
| UV filter | No | No |
| Powers fitted (D) | 0.25 to −6.75 | +0.50 |
| Back optic zone radius (mm) | 7.00 to 9.00 | 7.90 to 8.90 |
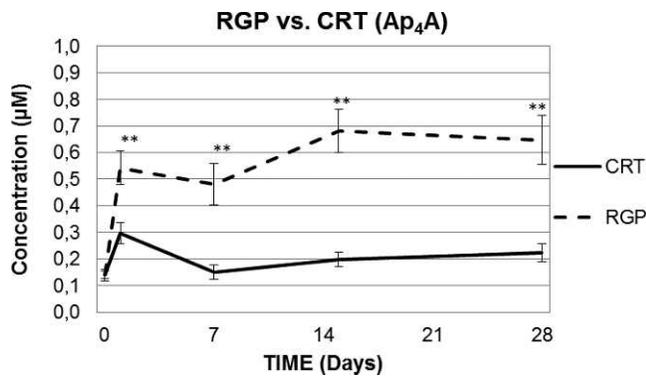
**FIGURE 1.** Concentrations of $Ap_4A$ during 1-month follow-up, with both RGP lenses and CRT lenses. $Ap_4A$ increases its concentration in tears in alignment-fit RGP lenses throughout the study ($P < 0.001$). For CRT lens wear, $Ap_4A$ concentrations show an increase in the first day and at the end of the study ($P = 0.041$). **$P$ value $< 0.05$ RGP versus CRT. Student's *t*-test.

irritation, grittiness, scratchiness, foreign body sensation, burning, light sensitivity, and itching. The patients had to choose between four different frequencies (1 = rarely; 2 = sometimes; 3 = frequently; and 4 = constantly).

## Tear Preparation and HPLC Analysis

After thawing, the samples were strongly vortexed for 5 minutes. The strips were carefully rinsed, and the liquid in the Eppendorf tube was heated in a 100°C bath for 20 minutes to precipitate proteins. In order to pellet the proteins, the tubes were centrifuged at 4000 rpm for 30 minutes. Diadenosine polyphosphates are not degraded by this treatment as previously demonstrated.[22] Supernatants were chromatographed through sample preparation cartridges (SEP-PAK Accell QMA; Waters Corp., Milford, MA) as previously described.[23] These eluents were injected at a volume of 10–100 µL.

Determination and quantification of diadenosine polyphosphates were performed by HPLC. The chromatographic system consisted of an isocratic pump (Waters 1515 Isocratic HPLC Pump; Waters Corp.), a dual absorbance detector (Waters 2487 Detector; Waters Corp.) and a sample injector valve (Rheodyne; Waters Corp.), all managed by HPLC software (Breeze 2 HPLC; Waters Corp.). The column (Nova-Pak C18; Waters Corp.) was 15 cm in length, 0.4 cm in diameter. The system was equilibrated overnight with the following mobile phase: 0.1 M $KH_2PO_4$, 2 mM tetrabutylammonium, 17% acetonitrile, pH 7.5.[16] Detection was monitored at 260-nm wavelength. All the peaks identified as putative dinucleotides were taken for phosphodiesterase treatment. Phosphodiesterase from *Crotalus adamanteus* (EC 3.1.4.1.; Sigma, St. Louis, MO), at a concentration of 1.0 U/mL, was incubated at room temperature for 30 minutes with the corresponding putative dinucleotide. The digestion products were analyzed by HPLC. Peaks were transformed into concentrations by means of external standards of known concentrations of diadenosine polyphosphates.

## Statistical Analysis

Data were analyzed by statistical software (SPSS version 15.0 for Windows; SPSS, Inc., Chicago, IL). The values presented are the means ± SEM of the experiments performed. Parametric tests were used to compare the study groups. Normality of distribution was assessed using the Shapiro-Wilk test. Differences between RGP lenses and CRT groups in tear function test were estimated by the Student's *t*-test for related samples. Study authors also used the Student's *t*-test for related samples to assess the differences among different follow-up visits into each group. Correlations between increments of $Ap_4A$ and tear volume
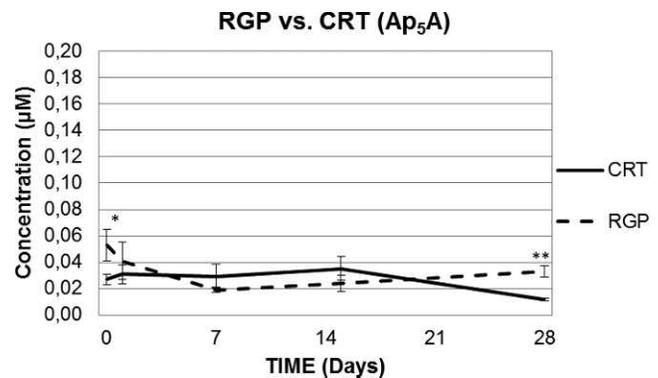


**FIGURE 2.** Concentrations of $Ap_5A$ during 1-month follow-up, with both RGP lenses and CRT lenses. $Ap_5A$ concentrations remained stable during the study in both groups, except at week 1 in RGP lenses ($P < 0.008$), and at the end of the study in CRT lenses ($P < 0.001$). *$P$ value $< 0.05$ RGP, **$P$ value $< 0.01$ versus CRT. Student's *t*-test.

regarding the preliminary visit were assessed by Pearson bivariate regression. A value of $P < 0.05$ was considered statistically significant.

## RESULTS

### Diadenosine Polyphosphates

$Ap_4A$ increased its concentration in tears in both alignment-fit RGP lenses and CRT in the first day after fitting, increasing 4- and 2-fold compared with the baseline visit, respectively. After 1 month, $Ap_4A$ continued significantly increased with alignment-fit lenses ($P < 0.001$). During CRT lens wear, $Ap_4A$ concentrations returned to baseline levels after 1 week, although a significant increase was also observed for samples collected 1 month after fitting ($P = 0.041$). Despite this, the values reached were much lower than during alignment-fit RGP lens wear. Indeed, there were statistically significant differences between RGP lenses and CRT lenses in $Ap_4A$ concentration in all follow-up visits ($P < 0.001$; Fig. 1). $Ap_5A$ concentrations remained stable during the study in both groups, except at week 1 in RGP lenses ($P < 0.008$), and at the end of the study in CRT lenses ($P < 0.001$; Fig. 2, Table 3).

### Schirmer I Test, Tear BUT (TBUT), and Corneal Staining

For alignment-fit RGP lenses, the tear volume increased slightly but not to a statistically significant level. In contrast, Schirmer was stable during the study with CRT lenses. BUT was stable during the study for both lenses (Figs. 3, 4, Table 3).

Corneal staining was similar with both types of lenses, without differences up to the end of the 1-month period for the alignment-fit RGP lens wearing. Conversely, significant differences were found from baseline at 2 weeks and 28 days of CRT wearing period ($P < 0.05$). Differences between RGP lenses and CRT were statistically significant at visit day 28 ($P < 0.001$; Fig. 5).

### Symptoms

Study authors evaluated the frequency of two typical dry eye-related symptoms such as discomfort and dryness. In the case of discomfort, it remained significantly increased during the whole period of the study with alignment-fit RGP lenses (Table 3). With CRT lenses, patients only show a statistically significant difference at visit day 28 ($P = 0.049$), but the levels of discomfort were significantly lower with CRT compared

TABLE 3.   Comparisons between Baseline and Different Follow-Up Visits and between Treatments for Different Variables during the Course of the Study

| Variable | Visit | RGP | P Value (against baseline) | CRT | P Value (against baseline) | P Value (RGP vs. CRT) |
|---|---|---|---|---|---|---|
| $Ap_4A$ (μM) mean (SEM) | Pre-visit | 0.140 (0.015) | | 0.139 (0.023) | | 0.971 |
| | 1 day | 0.543 (0.064) | <0.001 | 0.297 (0.039) | 0.001 | 0.002 |
| | 1 week | 0.481 (0.078) | <0.001 | 0.150 (0.027) | 0.758 | <0.001 |
| | 2 weeks | 0.681 (0.080) | <0.001 | 0.197 (0.127) | 0.108 | 0.002 |
| | 4 weeks | 0.647 (0.092) | <0.001 | 0.224 (0.033) | 0.041 | <0.001 |
| $Ap_5A$ (μM) mean (SEM) | Pre-visit | 0.053 (0.012) | | 0.027 (0.004) | | 0.046 |
| | 1 day | 0.041 (0.014) | 0.523 | 0.031 (0.007) | 0.620 | 0.523 |
| | 1 week | 0.019 (0.002) | 0.008 | 0.029 (0.009) | 0.817 | 0.284 |
| | 2 weeks | 0.024 (0.006) | 0.055 | 0.035 (0.009) | 0.367 | 0.315 |
| | 4 weeks | 0.033 (0.004) | 0.161 | 0.012 (0.001) | <0.001 | <0.001 |
| Schirmer (mm) mean (SEM) | Pre-visit | 18.15 (2.30) | | 15.91 (1.87) | | 0.454 |
| | 1 day | 21.12 (1.86) | 0.320 | 18.68 (2.33) | 0.359 | 0.418 |
| | 1 week | 19.5 (2.31) | 0.662 | 15.69 (2.10) | 0.939 | 0.229 |
| | 2 weeks | 22.23 (2.08) | 0.209 | 14.88 (2.06) | 0.715 | 0.016 |
| | 4 weeks | 21.80 (2.81) | 0.316 | 14.50 (2.00) | 0.610 | 0.040 |
| BUT (s) mean (SEM) | Pre-visit | 5.17 (0.28) | | 6.14 (0.59) | | 0.145 |
| | 1 day | 4.94 (0.35) | 0.607 | 6.15 (0.50) | 0.993 | 0.054 |
| | 1 week | 5.50 (0.52) | 0.545 | 6.83 (0.58) | 0.410 | 0.095 |
| | 2 weeks | 5.23 (0.59) | 0.926 | 6.41 (0.58) | 0.750 | 0.161 |
| | 4 weeks | 6.40 (0.78) | 0.112 | 6.14 (0.48) | 0.990 | 0.778 |
| Corneal staining mean (SEM) | Pre-visit | 0.71 (0.12) | | 0.74 (0.11) | | 0.855 |
| | 1 day | 0.88 (0.13) | 0.333 | 0.54 (0.12) | 0.217 | 0.061 |
| | 1 week | 0.69 (0.12) | 0.898 | 0.48 (0.11) | 0.090 | 0.204 |
| | 2 weeks | 0.69 (0.12) | 0.552 | 0.42 (0.11) | 0.042 | 0.105 |
| | 4 weeks | 1.0 (0.11) | 0.091 | 0.37 (0.10) | 0.016 | <0.001 |
| Discomfort mean (SEM) | Pre-visit | 0.64 (0.09) | | 0.58 (0.13) | | 0.706 |
| | 1 day | 2.40 (0.22) | <0.001 | 0.57 (0.15) | 0.960 | <0.001 |
| | 1 week | 2.66 (0.23) | <0.001 | 0.95 (0.16) | 0.078 | <0.001 |
| | 2 weeks | 1.77 (0.23) | <0.001 | 0.90 (0.18) | 0.159 | 0.005 |
| | 4 weeks | 1.90 (0.25) | <0.001 | 0.95 (0.13) | 0.049 | 0.002 |
| Dryness mean (SEM) | Pre-visit | 0.56 (0.12) | | 0.58 (0.15) | | 0.918 |
| | 1 day | 1.06 (0.19) | 0.029 | 0.47 (0.13) | 0.584 | 0.014 |
| | 1 week | 1.46 (0.24) | 0.002 | 0.85 (0.14) | 0.195 | 0.034 |
| | 2 weeks | 1.23 (0.23) | 0.017 | 1.00 (0.16) | 0.060 | 0.416 |
| | 4 weeks | 1.50 (0.23) | 0.001 | 1.05 (0.18) | 0.050 | 0.131 |

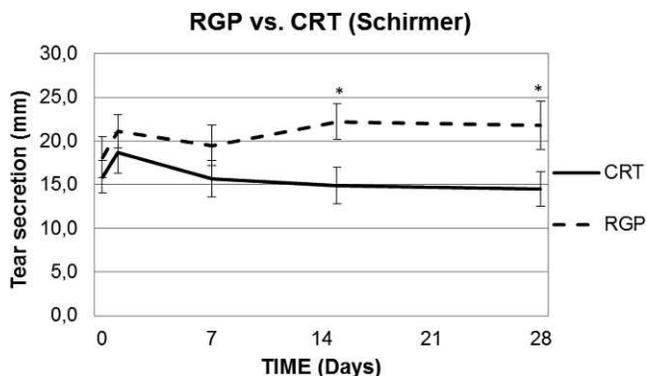Comparison post-wear with pre-wear values



FIGURE 3.   Schirmer test without anesthesia during 1-month follow-up, with both RGP lenses and CRT lenses. For RGP lenses, the tear volume increased slightly but not to a statistically significant level, and CRT lenses remained stable during the study. There are statistical differences between both wear regimens at the end of the study. *P value < 0.05 RGP versus CRT. Student's *t*-test.
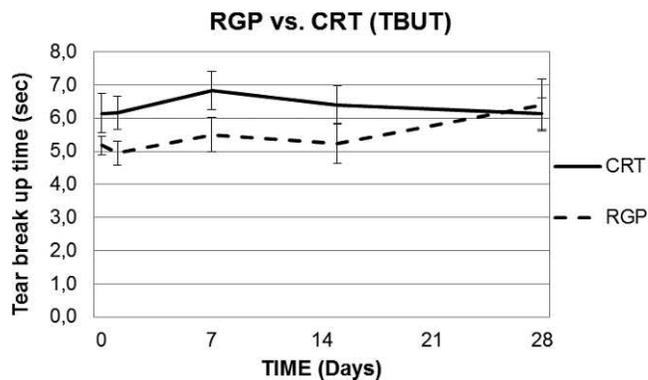


FIGURE 4.   TBUT during 1-month follow-up, with both RGP lenses and CRT lenses. There are no differences between both wear regimens or at follow-up visits.
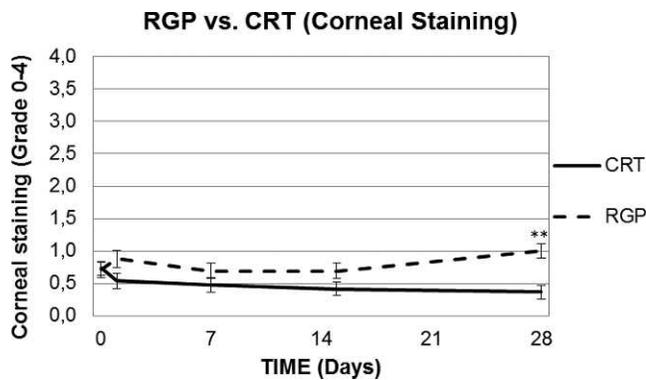
**RGP vs. CRT (Corneal Staining)**



FIGURE 5. Corneal staining grade during 1-month follow-up, with both RGP lenses and CRT lenses. There are no differences between both wear regimens. In RGP lens wear, it showed an increase at the end of the study. **$P$ value $< 0.01$ RGP versus CRT. Student's $t$-test.
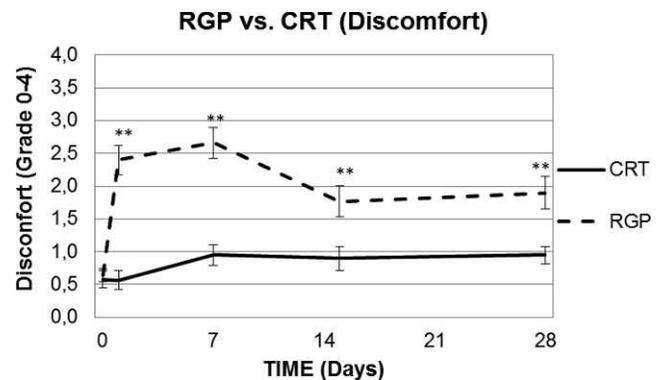
**RGP vs. CRT (Discomfort)**



FIGURE 6. Discomfort symptom frequency during 1-month follow-up, with both RGP lenses and CRT lenses. RGP daily wear regimen showed statistical differences with respect to CRT lenses in all follow-up visits. **$P$ value $< 0.01$ RGP versus CRT. Student's $t$-test.

with alignment-fit lenses. For dryness, the frequency was significantly increased during every study visit for alignment-fit RGP lenses. With CRT lenses, patients showed an increase in dryness frequency, but this was nonstatistically significant (Figs. 6, 7). When comparing both lenses, study authors observed differences only at day 1 and day 7 ($P = 0.014$ and $P = 0.034$, respectively).

## Correlations

The Pearson's correlation coefficient was 0.683 ($P = 0.029$) for tear volume and $Ap_4A$ differences at 15 days of RGP daily wear. The value of this coefficient indicates a moderate degree of correlation and the positive sign means that the greater the result of the Schirmer test, the greater the level of $Ap_4A$. With respect to the Pearson's correlation coefficient, both wear regimens showed a low-to-moderate positive correlation for all visits (Table 4).

## DISCUSSION

Nucleotides and dinucleotides are important tear molecules in normal and pathological conditions.[13,24–26] However, the possible changes of these substances in patients fitted with RGP lenses have not yet been investigated. With this aim, study authors evaluated $Ap_4A$ and $Ap_5A$ dinucleotide concentrations before and during 1 month with an alignment-fit RGP lens on a daily wear basis against reverse geometry CRT lenses worn overnight.

When the patients wore RGP lenses, there was an increase in $Ap_4A$ levels, whereas $Ap_5A$ remained stable. During the CRT wearing time, there was an increase in $Ap_4A$ during the first day, returning to baseline levels thereafter. $Ap_5A$ was stable throughout the study, and also during the wearing period of the latter type of lens.

Previous studies showed that the main mechanism of dinucleotide release in tears is caused by a mechanical stress on the epithelial cells of the ocular surface. It is also possible that $Ap_4A$ could be released as a consequence of mechanical corneal nerve stimulation.[13]

CL wear can induce a shear stress and neural stimulation on the corneal epithelium by blinking, and therefore, it seems clear that blinking during RGP lens wear increases the concentrations of $Ap_4A$.

Peral et al. found that when performing a mechanical stimulation of the corneal nerve fibers with a Belmonte esthesiometer with different intensities, $Ap_4A$ increases its

concentration but $Ap_5A$ remains constant. In contrast, both $Ap_4A$ and $Ap_5A$ increase their concentration by mechanical friction on the epithelium. The fact that $Ap_5A$ remains stable could indicate that part of the dinucleotide release is due to neural stimulation as a consequence of blinking. While the levels of $Ap_4A$ in the CRT group were similar to the ones obtained in previous experiments for the 12 blinks per minute rate,[13] this value was higher in the alignment-fit RGP group. This effect could be explained by the increased mechanical effect exerted by the RGP lens during the blinking. In the case of CRT lenses, there was no stimulation by lens friction because patients wore the lenses on an overnight regimen. A pilot experiment performed with six volunteers who slept with alignment-fit RGP lenses showed virtually the same concentrations in $Ap_4A$ to that obtained with the same individuals with CRT lenses (results not shown), confirming the effect of blinking in dinucleotides concentrations.

Tear volume was slightly increased when patients wore alignment-fit RGP lenses, but remained stable with CRT lenses. It has been demonstrated that dinucleotides can induce tear secretion,[15] and study results showed a positive correlation between increments of $Ap_4A$ and tear volume at follow-up visits. Therefore, an increase of $Ap_4A$ in RGP daily wear provides more tear volume. No significant differences were observed in TBUT and corneal staining during RGP and CRT wear time. However, TBUT was slightly lower and corneal staining was higher, reaching statistically significant differences
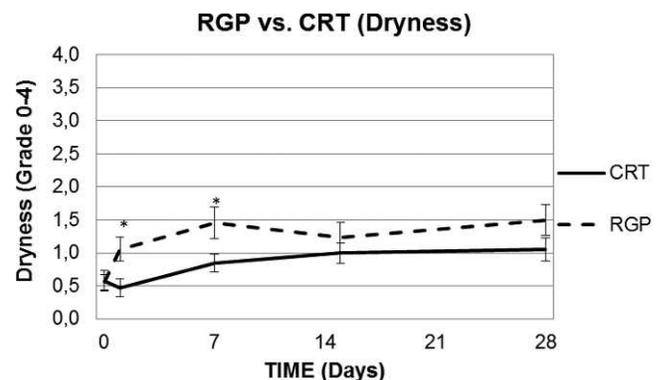
**RGP vs. CRT (Dryness)**



FIGURE 7. Dryness symptom frequency during 1-month follow-up, with both RGP lenses and CRT lenses. RGP daily wear regimen showed statistical differences with respect to CRT lenses in day 1 and 7 of lens wearing. *$P$ value $< 0.05$ RGP versus CRT. Student's $t$-test.

**TABLE 4.** Correlation between Diadenosine Tetraphosphates and Tear Volume Increments with Respect to the Preliminary Visit in Both Wear Regimens

| | $\Delta Ap_4A_{1d}$ vs. $\Delta Schirmer_{1d}$ | $\Delta Ap_4A_{1w}$ vs. $\Delta Schirmer_{1w}$ | $\Delta Ap_4A_{2w}$ vs. $\Delta Schirmer_{2w}$ | $\Delta Ap_4A_4w$ vs. $\Delta Schirmer_{4w}$ |
|---|---|---|---|---|
| **Alignment-fit RGP** | | | | |
| Pearson correlation | 0.589 | 0.535 | 0.683 | 0.566 |
| *P* value | 0.039 | 0.48 | 0.029 | 0.042 |
| **CRT lenses** | | | | |
| Pearson correlation | 0.555 | 0.563 | 0.572 | 0.508 |
| *P* value | 0.041 | 0.038 | 0.031 | 0.049 |

Significant correlation: $P < 0.05$ (2-tailed); that is, $\Delta Ap_4A_{2w} = Ap_4A_{pre} - Ap_4A_{2weeks}$; $\Delta Schirmer_{2w} = Schirmer_{previsit} - Schirmer_{2weeks}$.

at the end of the study in RGP wear time. This is probably due to the interaction of the contact lens with the tear film and blinking, such as an infrequent or partial blinking and/or thinning of prelens tear film.[27,28] Those results match with previous findings described by other authors.[29,30]

Discomfort was reported during the whole period of the study with both lenses. However, patients showed more discomfort with alignment-fit RGP lenses than with CRT lenses. One unexpected result is the increase in discomfort with CRT 'til the end of the first month, while remaining at a lower level than with alignment-fit RGP lenses. The blinking action could explain the discomfort reported by the patients during the first days of RGP lens daily wear, which was not experienced with CRT lenses worn overnight. The slight increase of discomfort with CRT lenses over time could be related to blinking in the morning during the period between the patients' awake and removal of the lenses. González-Méijome et al. demonstrated that in CRT wearers, discomfort was higher in the morning before removing than at night before fitting the lenses.[9]

From the clinical perspective, the paths of slight transient increase in $Ap_4A$ in the CRT group might reflect the initial discomfort reported by the patients at insertion and removal during the first days of treatment. Indeed, after the first day of treatment, the increase in $Ap_4A$ follows a similar path in alignment-fit RGP and CRT groups. This might reflect that the compression of the epithelium (which will be maximum during this period) does not increase significantly the expression of this dinucleotide still remaining at a lower rate compared with the RGP group.

Despite the growth of orthokeratology in clinical practice during the past decade, no study has directly addressed the question of signs and symptoms of dryness in CRT wearers. A similar situation lacking comparison terms is applicable to alignment-fit RGP lenses,[31,32] because comfort-related questions have been marginally considered in recent clinical research, probably because of their minor role in current fitting strategies. However, there are studies about soft contact lens wearers and dry eye symptomatology that showed an increase of dryness and discomfort.[5,33,34]

The dry eye symptomatic subjects with normal tear secretion presented similar $Ap_4A$ concentrations, which were found in the present study during RGP wearing time on a daily wear basis.[13] Jansen et al. found that blinking frequency with contact lens wear rose, while ocular irritation and discomfort increased during near tasks.[35] This suggests that an increase in discomfort produces a higher frequency of blinking, which causes the increased release of $Ap_4A$ to the tear film in order to minimize the symptoms. It would be interesting to conduct a long-term study with successful RGP patients to assess possible changes in the concentration of $Ap_4A$, once the patients report comfort rates comparable to soft contact lenses after a period of 3 to 6 months.[8]

Present results are significant to better understand the clinical response of the patient in terms of symptoms and signs during orthokeratology treatment in comparison with a model of daily RGP lens wear. Further research must be done in order to capture the actual effect of CRT over $Ap_4A$ expression by measuring $Ap_4A$ levels at different time intervals during the day, and also for longer periods in the medium and long-term of the orthokeratology treatment.

In conclusion, daily wear of an alignment-fit RGP lens increases the levels of $Ap_4A$ up to six times over baseline values, probably due to mechanical stimulation of the corneal epithelial cells, and this is associated with an increase in the levels of discomfort and dryness sensation reported by the patient. These effects might drive an increase in tear volume to minimize the symptomatology. Conversely, overnight orthokeratology lenses only increase $Ap_4A$ concentration during the very initial period of wear, although to a much lower extent than the alignment-fit lens worn on a daily basis, indicating that orthokeratology in the short term does not produce symptoms or signs of ocular dryness.

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