REVIEW

TITLE: Tear film osmolarity diurnal variations on the ocular surface review

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ABSTRACT

The measurement of tear film osmolarity has been suggested as a gold standard in the diagnosis of dry eye. Many tear film physiological variables oscillate during the day, therefore previous reports have studied how this variable changes throughout the day. The present brief review article will summarize the current clinical knowledge and main conclusion of the diurnal osmolarity variation in the tear film. For this purpose, the review performs a critical analysis on sample size and characteristics, the differences in the diurnal osmolarity variation on healthy versus altered tear film conditions or environment eyes, and time of day and number of measurements done. A total of twenty-one studies where one of the main objectives was to analyse the variance of the tear film osmolar value along different time-points in a day on a human cohort were compared. Osmolar tear film value does appear to be somewhat influenced by the time of day in healthy subjects and patients with ocular surface disease or altered by environmental conditions. Both, healthy and no-physiological tear film stable cohorts, showed variations in results depending on the study: no variations during the day or statistical different values at some point in the day (these differences could be in the middle of the day or between the beginning and the end of the day, with higher values in the morning than in the afternoon, or even the opposite situation). The possibility of diurnal variations in tear film osmolarity should be considered by the clinician since the time of day when the tear film measurements are made can be critical in making the right diagnosis. Future studies in the diurnal variation field may have to use a well-established range of measurement time-points and a larger group of healthy and tear film altered by pathological or environmental conditions subjects.

The tear film is a highly specialized structure that covers the anterior conjunctivae and cornea, being essential to maintaining a healthy and functional visual system.[1] It is a matrix-like structure composed of water, electrolytes, immunoglobulins, antimicrobial molecules and mucins, with a total volume of 7.0 ± 2.0 µl[2,3] distributed over two main tear film layers: an outer lipid layer composed of lipids, and a second layer between the outer layer and the cornea composed by an aqueous-mucin gradient.[4,5] Tear film dynamics is a highly complex process; good balance in its production, preservation and elimination, is vital to be able to fulfil its numerous roles and for the ocular surface health.[6,7] Despite this, diurnal variations in tear film variables have not yet been established. Many eye and tear film physiological variables fluctuate throughout the day, such as intraocular pressure,[8] corneal sensitivity,[9] tear pH,[10] the tear film stability,[11-13] or tear film meniscus.[14] In addition, those changes may be reflected in the symptomatology or the visual function experienced by patients (usually better in the morning and decline towards the evening).[4,15] The possibility of diurnal variations in tear film variables should be taken into account by the clinician since the time of day when measurements are performed can be critical in making the right diagnosis.[15]

Osmolarity in the dry eye

Osmolarity is defined as the total number of dissolved solute particles in one kilogram of a solution, without consideration of the nature of the particles, that is, their shape, size, density, configuration, or charge.[6] “Osmolality” (mOsm/kg) and “osmolarity” (mOsm/l) are two terms that are often used interchangeably when referring to dilute solutions. Tears and other biological solutions are mostly composed of water and therefore the two terms can be similar (the difference between osmolarity and osmolality is less than 5%).[6,16] Consistent with the current literature in the field, the term “osmolarity” is used here.

The measurement of tear film osmolarity has been suggested as a gold standard in the diagnosis of dry eye disease (DED).[6,7] DED has recently been redefined by the Dry Eye Workshop II (DEWS-II)[4,5] as “*a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film, and accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles*.” Similar to the original DEWS report in 2007,[11] the DEWS-II report reaffirmed that along with tear film instability, elevated tear film osmolarity is considered to be a core mechanism in symptoms of DED and ocular surface damage in this disease, regardless of the underlying aetiology.[4,17] Hyperosmolarity in the tear film may occur as a result of increased evaporation or reduced aqueous secretion; both origins generate

a considerable impact on tear film dynamics and stability.[6,7,18,19] The increase in the concentration of proteins and electrolytes caused by a reduction in tear volume that initially irritates the ocular surface goes on to cause inflammation and subsequent damage in evaporative dry eye, as thinning of the lipid layer allows for increased evaporation.[18,19] Hyperosmolarity then induces apoptosis, which serves as pro-inflammatory stress and reduces the ability of mucin-like molecules to lubricate the ocular surface that can permanently damage it.[7,20]

It has been established that the normal tear film osmolarity value is around 299-301 mOsm/l while in DED it reaches values of 325-340 mOsm/l.[4,6,21-25] This variable is a way of capturing a single parameter in the status of the tear film,[26] thereby providing a powerful tool in the diagnosis of tear film problems. There are several suggested cut-off values in the literature, ranging the principal them from 312 mOsm/l (79-94.7% sensitivity, 92-93.7 specificity), 316 mOsm/l (59-73% sensitivity, 65-94% specificity) to 317 mOsm/l (78% sensitivity, 78% specificity), where a higher value represents the limit between healthy/early/mild forms of the disease and moderate/severe patients.[6,21-24,27,28]

In addition to the high values, DED subjects are characterized by an inherent tear film instability that generates high variability in tear osmolarity recordings over time.[29-31] Indeed, the variability of osmolarity or increasing variation with increasing value is a statistical characteristic of DED patients because of the heteroscedasticity and might be considered as a clinical indication of the loss of tear film homeostasis that occurs with dry eye.[32] Actually, there is some controversy in how the tear film osmolarity value changes throughout the day.[33-52] While diurnal variations in tear film parameters are important in the daily clinic, over the last four decades there have not been too many clinical studies focused on the study of osmolarity diurnal changes. It has been hypothesized that eye closure creates a hypo-osmotic environment generated by the reduction of tear evaporation, production, and drainage. In addition, previous studies have found variation in the evaporation rates upon awakening,[53,54] with later stabilization probably because the reflex tears, the tear film stability or tear meniscus height.[12-14,37,44] It is important to note that tear volume, evaporation, stability, and film osmolarity have shown a strong relationship.[42,55]

The main aim of this brief review is to summarize the current clinical knowledge, methodologies, results, and conclusions in the tear film osmolarity diurnal variation pattern. For this purpose, the present review performs a critical analysis on sample size and characteristics, the differences in the diurnal osmolarity variation on healthy versus altered tear film conditions or environment eyes, and time of day and number of measurements done on studies published by platforms validated by a peer-review

process. To accomplish this analysis, the present review is divided into three main sections: 1) Analysis of study designs employed on the osmolarity diurnal variation assessment, 2) Report of osmolarity diurnal variation results found by studies, and 3) Clinical interpretation and implication.

The mains terms used for the search process were osmotic diurnal variation, tear film diurnal variation, tear film osmolarity variation, diurnal tear film osmolarity, tear daytime variation and tear diurnal pattern. Only original studies until 2020 where one of the main objectives was the analysis of the variance in the tear film osmolar value along different time-points in one day on a human cohort were included.

1. Analysis of study designs employed on the osmolarity diurnal variation assessment

The comparison between the study protocols used by the different research analysed in the present review was focused on three central points of an experimental design for an assessment of clinical tear film osmolarity diurnal changes: 1) the number and time-point measurement sessions studied during a day, 2) the measurement principle and instrument employed, and 3) the group of subjects or sample characteristics and size analysed.

1.1 Measurement sessions

There is no consensus in the time-point for the measurement session (from 6:00 am[37] to 10:00 pm),[33] or also the interval between them (from 10-minute intervals[34] to four/six-hour intervals)[49] (Table 1). In addition, some studies performed all the measurements in the same day,[35,39,40,42,43,45,47,48,51,52,56] while others followed the progression over two days,[37,46,50] or they repeated the protocol two consecutive days,[38,41] three non-consecutive days with 2- to 5-day intervals,[49] or over a six-day period[36] (Table 1).

It is difficult to make a real comparison between osmolarity values when authors have not used the same measurement time or the same total range of hours. For example, two different studies which compare “morning vs. afternoon” did not take the measurements at the same time-point (from 6:00 to 12:00 am vs from 1:00 to 10:00 pm); probably, differences between time-point chosen by the authors, and therefore studies, could be generated by the different “habits” existing in different countries or centres (work timetables, number, and schedules of meals, etc.) which may influence the protocols.

1.2 Measurement principle and instrument

Several osmometers have been developed based on determining one of the four colligative properties of solutions, which are freezing point, boiling point, vapour pressure and osmotic pressure; few osmometer specifically designed for a clinical tear film osmolarity assessment are available on the market.[6,57] Osmometers used in tear and other solutions during osmolarity assessment are based on the principle that the addition of a solute alters the chemical characteristics of a solvent and leads to a change in one of its colligative properties.[6]

In the assessment of tear film osmolarity diurnal variation, the most common osmometer used was the TearLab (TearLab Corporation, San Diego, CA, USA),[58-61] an impedance electric osmometer specific for tear film osmolarity measurement (Table 1).[39-45,47,48,51,52,56] Nowadays, there are only two tear osmolarity assessment specific osmometers available on the market, the TearLab osmometer and the i-Pen osmometer, both based on the electric impedance principle. Electrical impedance measurement has been shown as an accurate and repeatable technique for dry eye diagnosis;[25,30,62,63] moreover, electrical impedance osmometers are more clinically accessible because do not take up as much space and do not require a large volume of tears on each measurement.

In the second position, the other way commonly used to analyse osmolarity diurnal variations were various osmometers based on the freezing point depression principle,[34-38,49,50] such as the Clifton Osmometer (Clifton Technical Physics, Hartford, NY),[22,64,65] the Advanced Tear Osmometer (Advanced Instruments Inc., Norwood, MA),[66,67] the Fiske 210 osmometer (Advanced Instruments Inc., Norwood, MA)[68,69] or the Multi-OSMETTE 2430 osmometers (Precision Systems, Inc., Natick, MA, USA)[46,50] (Table 1). Except for the Advanced Tear Osmometer[66,67] (no longer available on the European market) in the Dalton et al. study,[38] none of those osmometers are specific to tear film osmolarity clinical assessment (all of them were regular laboratory osmometers).[34,35,37,46] Classically, freezing point depression was the most common method used for measuring osmolarity.[6,22,33-37,60,70] However, as stated earlier, there is no longer a tear specific osmometer available based on this principle. Even though it also has shown high repeatability in non-tear specific osmometers available based on freezing point depression techniques, some of them have shown lower values than those commonly reported in the literature.[49,68,69,71] There is some controversy in the relationship between the values obtained by both techniques, electrical impedance and freezing point depression based osmometers: some studies reported a perfect relationship between them,[22,62] while others found significant differences in their results.[68,69] Hence, it is difficult to perform a direct comparison between the results

obtained in studies using these two different techniques. On the other hand, electric impedance showed to be equivalent to vapour pressure measurements.[63]

On the tear film osmolarity daytime variation, only one study, Terry and Hill,[33] have used a vapour pressure osmometer (osmometer type and brand non mentioned in the publication) (Table 1).

Not all the devices employed in the different research studies used the same amount of tear sample; those differences could imply some errors in the final value because of the collection, dilution, or evaporation processes during a measurement.[58,68,71]This may generate some differences in the final results of the studies and a limitation for a real comparison between exact values depending on the device employed.[22,62,68]

1.3 Sample size and characteristics and size

The sample size studied shows a high variation between studies: from only two subjects in a study done in the year 1986,[36] to a higher sample on studies performed in the last fifteen years where groups of forty-fifty participants could be found (Table 1).[38,40,45,51] On those late studies, samples were divided into same-sized groups of healthy and ocular surface disease or under environmental alteration conditions participants (Table 1).[38,40,45,51] An exception to this "higher sample trend" was a study from 2020 where only 10 subjects (4 healthy, 4 DED and 2 CLW participants) were enrolled.[52] A larger population study, both healthy and pathological/environmental tear film altered subjects, may be required to detect a true daily osmolarity pattern and the differences between the tear osmolarity of dry eye subjects and healthy subjects. The DEWW-II report in the "Diagnostic Methodology" chapter recommends a minimum sample of 15 participants per group on tear film osmolarity assessment studies in order to obtain reliable study results.[4] The sample size used may be probably limited in all the studies by the difficulty to enrol participants from the beginning to the end of one or more days: the investigator should observe or watch over all subjects throughout the study period to avoid any activity that could influence the ocular surface and tear film (such as a video device use, reading a book, or sports and other activities that induce excessive sweating or stimulate the autonomic nervous system).[72]

It has been established that healthy and pathological subjects show different tear osmolarity values.[6,21-24,27,46,73] In diurnal osmolarity variations assessment, most of the studies were focused on healthy subjects (healthy or non-contact lens wearers [non-CLW]),[33-38,41,44,46,49,52,56] and less in subjects with altered tear film conditions or environment (pathological, contact lens wearers [CLW] or in biological stress situation subjects) (Table 1).[39,40,42,45-48,50-52]

Another important characteristic to consider when a study sample group is designed on any kind of tear film research study should be avoided to the gender- or age-related changes.[74,75] Whereas most of the studies are focused on subjects between 20 and 40 years old (young adults and adults),[33-37,40,42,44,46,47,49,50,56] not all of them made a point if the studied subjects were young, adults or older participants.[38,39,48,51] It has been reported that tear film function declines throughout life, and test results change with the advancing of age, especially those related to tear film stability (the other core mechanism in DED together with tear osmolarity), osmolarity and evaporation.[74,76,77] Only one study, Lee et al.,[50] used a one-gender group, a sample group of 20 males, the rest used women/men in a mixed sample; in some cases, the number of women/men sampled is very unbalanced, with a higher number of women than men.[45,49] On the other hand, gender was commonly related to dry eye status, where women are more likely to report dry eye symptoms or problems than men (steroid hormones play an important role in the ocular surface equilibrium and function).[78,79]

2. Osmolarity diurnal variation results found by the compared studies

As a general profile, both healthy subjects and participants with altered tear film conditions by pathologies or environmental situations osmolarity values obtained in the reviewed studies were in concordance with those reported in the relevant review publications and the DEWS report[4,6,21-24,27] (mean ± SD values = from 285 ± 2.4 to 331 ± 3.7 mOsm; Tables 2 and 3). Only one study, Garcia et al.,[49] reported lower values than the others,[33-47,50,51] or the values widely assumed by the literature as "common"[4,6,21-24,27] (mean ± SD = 270.00 ± 4.4 mOsm/kg; Tables 2 and 3). As the authors of this study explained in their discussion section, this may be caused by the technique employed for tear sample collection (capillary tube instead of a Whatman strip or a directly take through capillary action by a probe of a tear specific osmometer), the dilution and the frozen point depression process used, or the participant's age and sex (17 young females in a total group of 24 subjects). It is important to note that the low values reported in this study were similar to those obtained with a similar procedure (tear sample collected by a capillary tube and measured by freezing point depression osmometer non-tear specific) in three other recent studies where non-diurnal variation was analysed.[68,69,71]

2.1 Studies that do not report a difference in tear film osmolar diurnal variation

Most of the studies healthy subjects were enrolled and did not show variation in the diurnal osmolarity pattern (diurnal values between 285 ±2.4 and 310 ± 5.7 mOsm, Table 2);[33,35,37-39,41-43,45,49-51] it has been reported that consecutive measurements of the

tear film osmolarity of healthy subjects in short periods showed a lower variability, contrary to those measurements made in DED.[25,80] In addition, when an inter-day analysis was performed on healthy studies found no significant differences in the mean value obtained at the same point-time between different days over consecutive (303.2 ±4.8 vs 302.9 ±3.1 mOsm at 2:00 pm)[50] and non-consecutive[49] days (mean value from the six visits 270 ±4.4 mOsm).

Like in healthy cohorts, subjects that are pathological or in physiological stress (DED, medicated or sleep-deprived subjects) also showed some variations in results depending on the study. Contrary to the healthy cohort, few studies showed no variations during the day in subjects with an altered tear film by conditions or environment (diurnal values between 304 ±10.8 and 319.65 ±15.8 mOsm, Table 3).[42,45] It is important to note that values reported on those cohorts of subjets by the different studies were in most of the cases over those values commonly used as cut-off criteria to differentiate healthy and pathological subjects - 312, 316 or 317.[6,22,23,55] Similar situation as occurring with pathological subjects was reported in CLW (with and without keratoconus), with no variation between the morning and afternoon in some studies (diurnal values between 281 ±7.07 and 340 ±15.6 mOsm, Table 3).[39,40,48,51]

2.2 Studies that report a difference in tear film osmolar diurnal variation

Some studies where healthy subjects were enrolled found show variation in the diurnal osmolarity pattern, where half of them were performed after 2005 or without an specific designed to tear film assessment osmometer (diurnal values between 296.48 ±12.9 and 318 ±31 mOsm, Table 2);[34,36,38,42,46,56] when differences between some measurement points were found, these could be in the middle of the day;[42] or between the beginning and the end of the day, with higher values in the morning than in the afternoon,[38] or even the opposite situation (Table 2).[34,38,46] Similar to healthy subjects, different values at some point in the day were reported in pathological or in biological stress situation subjects (diurnal values between 295.5 ±6.2 and 331 ±3.7mOsm, Table 3).[42,46,50] However, in contrasts to the healthy subjects, pathological patients showed variations in the inter-day analysis (300.1 ±3.8 vs. 302.2 ±4.5 at 2:00 pm).[50] Dry eye is characterized by an inherent tear film instability that generates high variability in tear osmolarity recordings over time.[29-31] DED subjects show higher tear variation over time than observed in healthy subjects on consecutive measurements.[25,30,80] This variability could be found in inter-eye difference, which is correlated with disease severity and has been proposed as a diagnostic indicator of this disease.[29,31,81,82]

The same situation as occurs with pathological subjects was reported in CLW, with different values at some point in the day (diurnal values between 292.2 ±5.4 and 340

±15.6 mOsm, Table 3).[40,47,51] The insertion of contact lenses leads to an initial reduction in tear film osmolality followed by a hyperosmolarity phase,[6,48] and it can be assumed that different contact lenses materials with different chemical properties or hydrations may have a different impact on the tear film osmolarity.[39,40,47,48,51] In addition, the osmolality of soft contact lens solutions or blister packaging solutions may also play a role in the tear film hyperosmolarity,[83-85] an increment in the osmolarity value that could be more relevant when storage case or multipurpose solutions are not used properly.[86-88]

2.3 Studies that compare detail the various conditions

Although there is some controversy over diurnal tear film osmolarity, the osmolarity variable value has been observed to differ between healthy individuals and those pathological or in a "non-physiological balanced situation". Some of the reviewed studies also made a comparison of the results obtained in the healthy subjects and participants with an altered tear film by conditions or environment at each time point, reporting different findings. One study reported daily variation only in the healthy group and not in the DED participants, and found no significant differences between both groups at any of the time-points analysed.[42] On the other hand, another study that reported no variation in any moment of the day, found statistically significantly higher values in pathological subjects than those in the control group [45,50] (Tables 2 and 3). No statistical differences were found between CLW versus non-CLW for the morning (values between 292.9 ± 6.1 and 303.32 ± 10.41 mOsm/l on CLW while between 292.1 ± 6.1 and 296.90 ± 8.51 mOsm/l on non-CLW) or afternoon readings (values between 293.9 ± 6.3 and 300.10 ± 10.51 on CLW while between 292.3 ± 4.1 and 298.90 ± 8.71 mOsm/l on non-CLW),[39,51] whereas there are statistical differences on the midday time-point (values between 293.9 ± 6.3 and 294.2 ± 6.2 on CLW while between 292.3 ± 4.1 and 293.3 ± 4.9 mOsm/l on non-CLW)[51] (Tables 2 and 3).

3. Clinical interpretation and implication

On the present revision, it can be found studies which report that upon awakening, osmolarity is in its lower values,[33,37,39,44,46] but reaches a nearly stable profile during the day within a few minutes (Table 2). This may have a clinical implication such clinicians should avoid performing measurements right before awakening or wait near to 40-60 min to use values for diagnosis. It has been hypothesized that eye closure creates a hypo-osmotic environment generated by the reduction of tear evaporation, production, and drainage.[37,44] Because of that, tear film osmolarity is in its lower values upon eyelid opening.[33,37,44] Then, in the afternoon it rises to "standard" values[33-35,37-39,41,42,44,45,50]

caused by an increase in evaporation (suggested as the main cause for tear film thinning and volume decreasing)[18,89-91] as the eye responds to the relative variations on the surrounding conditions. In concordance with this theory, Tomlinson and Cedarstaff[53] found that in healthy patients' evaporation is at its lowest immediately upon waking, then rises rapidly within the first 2 hours, and remains constant for the next 12 hours in a fairly narrow range of evaporation rate values. Similar results were found by Wojtowicz et al.[54] on aqueous tear evaporation in healthy subjects who concluded that evaporation rates at the two studied day-points were essentially equal. Tomlinson and Cedarstaff[53] also attributed this initial low tear evaporation upon awakening profile to a low human tear production on awakening and/or tear stability being high at this time because of a thick lipid layer. Therefore, it is expected to find higher osmolarity values in the afternoon than in the morning, even in healthy individuals with regular tear values due to greater opportunity for evaporation from the tear film with an increasing number of waking hours.[35] Healthy and DED subjects have reported that many symptoms worsened over the day within two hours of getting up in the morning and at the end of the day, suggesting an environment- or task-related aetiology for dry eye symptoms.[92,93]

Another explanation proposed by Niimi et al.[44] for these lower values at the beginning of the day and the later stabilization is the reflex tears induced by eye-opening. Reflex tearing occurs upon awakening as a protective mechanism to wash out unwanted substances accumulated during overnight eye closure.[94] This reflex leads to an increase in tear meniscus height (TMH) immediately after eye-opening in the morning that recovers to baseline approximately 30 minutes after awakening.[14] Tear meniscus is directly related to the total tear film volume since it has been estimated that it holds 75-90% of the total tear film volume.[42,95-97] Tear meniscus and tear film osmolarity have shown a strong relationship.[55] Li et al.[42] analysed the diurnal variation in the osmolarity and different tear meniscus parameters in DED and healthy subjects, and both variables showed a near to stable profile and a significant correlation between them. In concordance with these results, Lira et al.[98] found in healthy subjects no statistical differences in the TMH parameter and another volume test (Schirmer test) between the morning and evening measurements. In addition to all of these results, in the Montana reports on CLW,[47,48] it is reported that TMH values vary in the same direction as the osmolarity throughout the day: when osmolarity raises the TMH tends to decrease, and when the osmolarity goes down the THM increase. This data shows that in the healthy subject the whole tear system can compensate for changes in the environment to maintain a very stable osmolarity. However, in dry eye subjects, that ability to compensate is lost and the osmolarity rises concurrently with epithelial stress and

inflammation. From a clinical point of view, similar to previous purposes about inter-eye differences or the variation of consecutive measurements in a small period of time as indicators of tear film physiological instability (e.g., DED condition),[4,21,30,31] inter- or intra-day variations may be used as a diagnostic indicator to differentiate between healthy and no-healthy tear film.

In addition to volume or evaporation rate, the other core mechanism of the DED in close relationship with the osmolarity is the tear film stability.[11] Patel et al.[12] examined tear film stability using a keratometer in healthy young subjects (2-hour intervals between 8:00 am and 8:00 pm 9 times per session) and found in concordance with the osmolarity that at the beginning of the day tear film stability starts at lower values but quickly stabilizes for the rest of the day. Similar results were found by Walker et al.[13] with the tear film break-up test (BUT) in patients with DED in two 6 hours apart sessions, Bitton et al.[99] with non-invasive break-up time (NIBUT) in healthy subjects into a four-session two days apart protocol, and Pena-Verdeal et al.[100] with BUT in healthy patients along a four-session protocol for one day: there were no significant differences in tear film stability between morning and evening measurements. This instability at the beginning of the day may be related to the high evaporation rates, and therefore, hyperosmolarity.

CONCLUSION

In summary, tear film osmolarity does appear to be somewhat influenced by the time of day in healthy and tear film altered by environment or pathologies patients. Both, healthy and no-physiological tear film stables cohorts, showed variations in results depending on the study: no variations during the day, or statistical different values at some point in the day; these differences could be in the middle of the day or between the beginning and the end of the day, with higher values in the morning than in the afternoon, or even the opposite situation. To prevent some influence on these little diurnal variations, clinicians may take the osmolarity measurements at the same time of day in the following session to be able to compare the values between patients. Future studies in the diurnal variation field may have to use a well-established range of measurement time-points and a larger group of healthy and no-healthy subjects.

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REFERENCES

1 Korb DR. The tear film-its role today and in the future. In: Butterworth-Heinemann ed. *The tear film, structure, function and clinical evaluation*, 2002. p 126-192.

2 Ehlers N, Kessing SV, Norn MS. Quantitative amounts of conjunctival mucous secretion and tears. *Acta Ophthalmol (Copenh)* 1972; 50: 210-214.

3 Mishima S, Gasset A, Klyce SD, Jr. et al. Determination of tear volume and tear flow. *Invest Ophthalmol* 1966; 5: 264-276.

4 Wolffsohn JS, Arita R, Chalmers R et al. TFOS DEWS II Diagnostic Methodology report. *Ocul Surf* 2017; 15: 539-574.

5 Craig JP, Nichols KK, Akpek EK et al. TFOS DEWS II Definition and Classification Report. *Ocul Surf* 2017; 15: 276-283.

6 Stahl U, Willcox M, Stapleton F. Osmolality and tear film dynamics. *Clin Exp Optom* 2012; 95: 3-11.

7 Liu H, Begley C, Chen M et al. A link between tear instability and hyperosmolarity in dry eye. *Invest Ophthalmol Vis Sci* 2009; 50: 3671-3679.

8 Pointer JS. The diurnal variation of intraocular pressure in non-glaucomatous subjects: relevance in a clinical context. *Ophthalmic Physiol Opt* 1997; 17: 456-465.

9 du Toit R, Vega JA, Fonn D et al. Diurnal variation of corneal sensitivity and thickness. *Cornea* 2003; 22: 205-209.

10 Carney LG, Hill RM. Human tear pH. Diurnal variations. *Arch Ophthalmol* 1976; 94: 821-824.

11 The definition and classification of dry eye disease: report of the Definition and Classification Subcommittee of the International Dry Eye WorkShop (2007). *Ocul Surf* 2007; 5: 75-92.

12 Patel S, Bevan R, Farrell JC. Diurnal variation in precorneal tear film stability. *Am J Optom Physiol Opt* 1988; 65: 151-154.

13 Walker PM, Lane KJ, Ousler GW, 3rd et al. Diurnal variation of visual function and the signs and symptoms of dry eye. *Cornea* 2010; 29: 607-612.

14 Shen M, Wang J, Tao A et al. Diurnal variation of upper and lower tear menisci. *Am J Ophthalmol* 2008; 145: 801-806.

15 Guillon M, Shah S. Rationale for 24-hour management of dry eye disease: A review. *Cont Lens Anterior Eye* 2019; 42: 147-154.

16 Erstad BL. Osmolality and osmolarity: narrowing the terminology gap. *Pharmacotherapy* 2003; 23: 1085-1086.

17 Nelson JD, Craig JP, Akpek EK et al. TFOS DEWS II Introduction. *Ocul Surf* 2017; 15: 269-275.

18 King-Smith PE, Ramamoorthy P, Braun RJ et al. Tear film images and breakup analyzed using fluorescent quenching. *Invest Ophthalmol Vis Sci* 2013; 54: 6003-6011.

19 Braun RJ, King-Smith PE, Begley CG et al. Dynamics and function of the tear film in relation to the blink cycle. *Prog Retin Eye Res* 2015; 45: 132-164.

20 Sullivan BD, Crews LA, Messmer EM et al. Correlations between commonly used objective signs and symptoms for the diagnosis of dry eye disease: clinical implications. *Acta Ophthalmol* 2014; 92: 161-166.

21 Jacobi C, Jacobi A, Kruse FE et al. Tear film osmolarity measurements in dry eye disease using electrical impedance technology. *Cornea* 2011; 30: 1289-1292.

22 Tomlinson A, McCann LC, Pearce EI. Comparison of human tear film osmolarity measured by electrical impedance and freezing point depression techniques. *Cornea* 2010; 29: 1036-1041.

23 Messmer EM, Bulgen M, Kampik A. Hyperosmolarity of the tear film in dry eye syndrome. *Dev Ophthalmol* 2010; 45: 129-138.

24 Masmali A, Alrabiah S, Alharbi A et al. Investigation of tear osmolarity using the TearLab Osmolarity System in normal adults in Saudi Arabia. *Eye Contact Lens* 2014; 40: 74-78.

25 Gokhale M, Stahl U, Jalbert I. In situ osmometry: validation and effect of sample collection technique. *Optom Vis Sci* 2013; 90: 359-365.

26 Muselier-Mathieu A, Bron AM, Mathieu B et al. Ocular surface assessment in soft contact lens wearers; the contribution of tear osmolarity among other tests. *Acta Ophthalmol* 2014; 92: 364-369.

27 Tomlinson A, Khanal S, Ramaesh K et al. Tear film osmolarity: determination of a referent for dry eye diagnosis. *Invest Ophthalmol Vis Sci* 2006; 47: 4309-4315.

28 Khanal S, Tomlinson A, McFadyen A et al. Dry eye diagnosis. *Invest Ophthalmol Vis Sci* 2008; 49: 1407-1414.

29 Potvin R, Makari S, Rapuano CJ. Tear film osmolarity and dry eye disease: a review of the literature. *Clin Ophthalmol* 2015; 9: 2039-2047.

30 Keech A, Senchyna M, Jones L. Impact of time between collection and collection method on human tear fluid osmolarity. *Curr Eye Res* 2013; 38: 428-436.

31 Lemp MA, Bron AJ, Baudouin C et al. Tear osmolarity in the diagnosis and management of dry eye disease. *Am J Ophthalmol* 2011; 151: 792-798 e791.

32 Sullivan B. Challenges in using signs and symptoms to evaluate new biomarkers of dry eye disease. *Ocul Surf* 2014; 12: 2-9.

33 Terry JE, Hill RM. Human tear osmotic pressure: diurnal variations and the closed eye. *Arch Ophthalmol* 1978; 96: 120-122.

34 Benjamin WJ, Hill RM. Human tears: osmotic characteristics. *Invest Ophthalmol Vis Sci* 1983; 24: 1624-1626.

35 Farris RL, Stuchell RN, Mandel ID. Tear osmolarity variation in the dry eye. *Trans Am Ophthalmol Soc* 1986; 84: 250-268.

36 Benjamin WJ, Hill RM. Tear osmotic differences across the ocular surface. *Graefes Arch Clin Exp Ophthalmol* 1986; 224: 583-586.

37 Gilbard JP, Cohen GR, Baum J. Decreased tear osmolarity and absence of the inferior marginal tear strip following sleep. *Trans Am Ophthalmol Soc* 1991; 89: 209-213; discussion 213-204.

38 Dalton K, Jones L. The performance of a novel nanolitre osmometer to investigate diurnal tear film osmolality. *Optom Vis Sci* 2005; 82.

39 Dimit R, Miller W, Leach N et al. Comparison of Comparison of morning and afternoon osmolarity in silicone hydrogel wearers and non-contact lens wearers. *Invest Ophthalmol Vis Sci* 2011; 52: 6538.

40 Montani G. Modification of the tear film osmolarity with the use of contact lenses in omafilcon A and methafilcon A materials. In. 3rd Global Specialty Lens Symposium (GSLS) Las Vegas 2011.

41 Khanal S, Millar TJ. Barriers to clinical uptake of tear osmolarity measurements. *Br J Ophthalmol* 2012; 96: 341-344.

42 Li M, Du C, Zhu D et al. Daytime variations of tear osmolarity and tear meniscus volume. *Eye Contact Lens* 2012; 38: 282-287.

43 Oncel BA, Pinarci E, Akova YA. Diurnal variation of the tear osmolarity in normal subjects measured by a new microchip system. *Eur J Ophthalmol* 2012; 22 Suppl 7: S1-4.

44 Niimi J, Tan B, Chang J et al. Diurnal Pattern of Tear Osmolarity and Its Relationship to Corneal Thickness and Deswelling. *Cornea* 2013; 32: 1305-1310.

45 Yiğit U, Evcimen Y, Kırık F et al. Daytime variations of tear osmolarity measurement in dry eyes patients (*article in turkish*). *Turkish Journal of Ophthalmology* 2013; 43: 437-441.

46 Kim D, Kim HJ, Hyon JY et al. Effects of oral mucolytics on tear film and ocular surface. *Cornea* 2013; 32: 933-938.

47 Montani G. Tear meniscus height, osmolarity and optical quality in silicone hydrogel contact lens wearers. In. 6th Global Specialty Lens Symposium (GSLS) Las Vegas 2014.

48 Montani G. Tear volume, osmolarity in keratoconus patients fitted with rigid gas permeable lenses. 6th Global Specialty Lens Symposium (GSLS); 2014 January, 23 to 26; Las Vegas

49 Garcia N, Teson M, Enriquez-de-Salamanca A et al. Basal values, intra-day and inter-day variations in tear film osmolarity and tear fluorescein clearance. *Curr Eye Res* 2014; 39: 673-679.

50 Lee YB, Koh JW, Hyon JY et al. Sleep deprivation reduces tear secretion and impairs the tear film. *Invest Ophthalmol Vis Sci* 2014; 55: 3525-3531.

51 Aslan Bayhan S, Bayhan HA, Muhafiz E et al. Effects of osmoprotective eye drops on tear osmolarity in contact lens wearers. *Can J Ophthalmol* 2015; 50: 283-289.

52 van Setten GB. Osmokinetics: Defining the Characteristics of Osmotic Challenge to the Ocular Surface. *Klin Monbl Augenheilkd* 2020; 237: 644-648.

53 Tomlinson A, Cedarstaff TH. Diurnal variation in human tear evaporation. In: Elsevier ed. Annual Meeting of the British Contact Lens Association, 1992. p 77-79.

54 Wojtowicz JC, McCulley JP. Assessment and impact of the time of day on aqueous tear evaporation in normal subjects. *Eye Contact Lens* 2009; 35: 117-119.

55 Garcia-Resua C, Pena-Verdeal H, Remeseiro B et al. Correlation between tear osmolarity and tear meniscus. *Optom Vis Sci* 2014; 91: 1419-1429.

56 Pena-Verdeal H, Vazquez-Sanchez C, Garcia-Queiruga J et al. Differences of tear film osmolarity between two time-points of the day in healthy subjects. *Asian Journal of Ophthalmology* 2020; 17: 173-181.

57 McGinnigle S, Naroo SA, Eperjesi F. Evaluation of dry eye. *Surv Ophthalmol* 2012; 57: 293-316.

58 Yoon D, Gadaria-Rathod N, Oh C et al. Precision and accuracy of TearLab osmometer in measuring osmolarity of salt solutions. *Curr Eye Res* 2014; 39: 1247-1250.

59 Benelli U, Nardi M, Posarelli C et al. Tear osmolarity measurement using the TearLab Osmolarity System in the assessment of dry eye treatment effectiveness. *Cont Lens Anterior Eye* 2010; 33: 61-67.

60 Sullivan BD, Whitmer D, Nichols KK et al. An objective approach to dry eye disease severity. *Invest Ophthalmol Vis Sci* 2010; 51: 6125-6130.

61 Eperjesi F, Aujla M, Bartlett H. Reproducibility and repeatability of the OcuSense TearLab osmometer. *Graefes Arch Clin Exp Ophthalmol* 2012; 250: 1201-1205.

62 Yoon D, Gadaria-Rathod N, Oh C et al. Precision and Accuracy of TearLab Osmometer in Measuring Osmolarity of Salt Solutions. *Curr Eye Res* 2014.

63 Rocha G, Gulliver E, Borovik A et al. Randomized, masked, in vitro comparison of three commercially available tear film osmometers. *Clin Ophthalmol* 2017; 11: 243-248.

64 White KM, Benjamin WJ, Hill RM. Human basic tear fluid osmolality. II. Importance of processing strategy. *Acta Ophthalmol (Copenh)* 1993; 71: 530-538.

65 White KM, Benjamin WJ, Hill RM. Human basic tear fluid osmolality. I. Importance of sample collection strategy. *Acta Ophthalmol (Copenh)* 1993; 71: 524-529.

66 Yildiz EH, Fan VC, Banday H et al. Evaluation of a new tear osmometer for repeatability and accuracy, using 0.5-microL (500-Nanoliter) samples. *Cornea* 2009; 28: 677-680.

67 Srinivasan S, Joyce E, Jones LW. Tear osmolality and ferning patterns in postmenopausal women. *Optom Vis Sci* 2007; 84: 588-592.

68 Pena-Verdeal H, Garcia-Resua C, Minones M et al. Accuracy of a Freezing Point Depression Technique Osmometer. *Optom Vis Sci* 2015; 92: e273-283.

69 Garcia N, Melvi G, Pinto-Fraga J et al. Lack of Agreement among Electrical Impedance and Freezing-Point Osmometers. *Optom Vis Sci* 2016; 93: 482-487.

70 Kohler MF, Marks JR, Wiseman RW et al. Spectrum of mutation and frequency of allelic deletion of the p53 gene in ovarian cancer. *J Natl Cancer Inst* 1993; 85: 1513-1519.

71 Pena-Verdeal H, Garcia-Resua C, Vazquez-Sanchez C et al. Reproducibility in measuring tear samples using a freezing point depression osmometer. *Clin Exp Optom* 2019; 102: 571-575.

72 Himebaugh NL, Begley CG, Bradley A et al. Blinking and tear break-up during four visual tasks. *Optom Vis Sci* 2009; 86: E106-114.

73 Willcox MDP, Argueso P, Georgiev GA et al. TFOS DEWS II Tear Film Report. *Ocul Surf* 2017; 15: 366-403.

74 Ozdemir M, Temizdemir H. Age- and gender-related tear function changes in normal population. *Eye (Lond)* 2010; 24: 79-83.

75 Afonso AA, Monroy D, Stern ME et al. Correlation of tear fluorescein clearance and Schirmer test scores with ocular irritation symptoms. *Ophthalmology* 1999; 106: 803-810.

76 Mathers WD, Lane JA, Zimmerman MB. Tear film changes associated with normal aging. *Cornea* 1996; 15: 229-234.

77 Lin PY, Tsai SY, Cheng CY et al. Prevalence of dry eye among an elderly Chinese population in Taiwan: the Shihpai Eye Study. *Ophthalmology* 2003; 110: 1096-1101.

78 Scuderi G, Contestabile MT, Gagliano C et al. Effects of phytoestrogen supplementation in postmenopausal women with dry eye syndrome: a randomized clinical trial. *Can J Ophthalmol* 2012; 47: 489-492.

79 Gagliano C, Caruso S, Napolitano G et al. Low levels of 17-beta-oestradiol, oestrone and testosterone correlate with severe evaporative dysfunctional tear syndrome in postmenopausal women: a case-control study. *Br J Ophthalmol* 2014; 98: 371-376.

80 Nolfi J, Caffery B. Randomized comparison of in vivo performance of two point-of-care tear film osmometers. *Clin Ophthalmol* 2017; 11: 945-950.

81 Caffery B, Chalmers RL, Marsden H et al. Correlation of tear osmolarity and dry eye symptoms in convention attendees. *Optom Vis Sci* 2014; 91: 142-149.

82 Szczesna-Iskander DH. Measurement variability of the TearLab Osmolarity System. *Cont Lens Anterior Eye* 2016; 39: 353-358.

83 Dalton K, Subbaraman LN, Rogers R et al. Physical properties of soft contact lens solutions. *Optom Vis Sci* 2008; 85: 122-128.

84 Menzies KL, Jones L. In vitro analysis of the physical properties of contact lens blister pack solutions. *Optom Vis Sci* 2011; 88: 493-501.

85 Montani G. Intrasubject tear osmolarity changes with two different types of eyedrops. *Optom Vis Sci* 2013; 90: 372-377.

86 Pinto-Fraga J, Abengozar-Vela A, Lopez-Miguel A et al. Effect of the osmolarity change in multipurpose solutions induced by an improper contact lens case cleaning procedure. *Cont Lens Anterior Eye* 2016; 39: 177-184.

87 Abengozar-Vela A, Pinto FJ, Gonzalez-Meijome JM et al. Contact lens case cleaning procedures affect storage solution pH and osmolality. *Optom Vis Sci* 2011; 88: 1414-1421.

88 Pena-Verdeal H, Garcia-Queiruga J, Garcia-Resua C et al. Osmolality and pH of commercially available contact lens care solutions and eye drops. *Cont Lens Anterior Eye* 2020.

89 King-Smith PE, Hinel EA, Nichols JJ. Application of a novel interferometric method to investigate the relation between lipid layer thickness and tear film thinning. *Invest Ophthalmol Vis Sci* 2010; 51: 2418-2423.

90 Kimball SH, King-Smith PE, Nichols JJ. Evidence for the major contribution of evaporation to tear film thinning between blinks. *Invest Ophthalmol Vis Sci* 2010; 51: 6294-6297.

91 Nichols JJ, King-Smith PE, Hinel EA et al. The use of fluorescent quenching in studying the contribution of evaporation to tear thinning. *Invest Ophthalmol Vis Sci* 2012; 53: 5426-5432.

92 Begley CG, Chalmers RL, Abetz L et al. The relationship between habitual patient-reported symptoms and clinical signs among patients with dry eye of varying severity. *Invest Ophthalmol Vis Sci* 2003; 44: 4753-4761.

93 Torricelli AA, Novaes P, Matsuda M et al. Correlation between signs and symptoms of ocular surface dysfunction and tear osmolarity with ambient levels of air pollution in a large metropolitan area. *Cornea* 2013; 32: e11-15.

94 Sack RA, Conradi L, Krumholz D et al. Membrane array characterization of 80 chemokines, cytokines, and growth factors in open- and closed-eye tears: angiogenin and other defense system constituents. *Invest Ophthalmol Vis Sci* 2005; 46: 1228-1238.

95 Holly FJ. Physical chemistry of the normal and disordered tear film. *Trans Ophthalmol Soc U K* 1985; 104 (Pt 4): 374-380.

96 Bron AJ, Tomlinson A, Foulks GN et al. Rethinking dry eye disease: a perspective on clinical implications. *Ocul Surf* 2014; 12: S1-31.

97 Yokoi N, Bron AJ, Tiffany JM et al. Relationship between tear volume and tear meniscus curvature. *Arch Ophthalmol* 2004; 122: 1265-1269.

98 Lira M, Oliveira ME, Franco S. Comparison of the tear film clinical parameters at two different times of the day. *Clin Exp Optom* 2011; 94: 557-562.

99 Bitton E, Keech A, Jones L et al. Subjective and objective variation of the tear film pre- and post-sleep. *Optom Vis Sci* 2008; 85: 740-749.

100 Pena-Verdeal H, Garcia-Resua C, Ramos L et al. Diurnal variations in tear film break-up time determined in healthy subjects by software-assisted interpretation of tear film video recordings. *Clin Exp Optom* 2016; 99: 142-148.

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693 **Table 1.** Summary of the principal protocols in tear film osmolarity diurnal variation analysis reported by the literature. VP: Vapour Pressure. FPD: Freezing
694 Point Depression. EI: Electric Impedance. LO: Laboratory Osmometer. TFO: Tear Film Osmometer. CLW: Contact Lens Wearer. KC: Keratoconus. SD:
695 Sleep-Deprived. *Measurement time not reported.
696

Year	Authors	Instrument			Study group	Measurement session
		Principle	Name	Design		
1978	**Terry and Hill** [33]	VP	No detailed	LO	6 healthy	1-hour interval from 9:00 am to 10:00 pm (five-day period)
1983	**Benjamin and Hill** [34]	FPD	No detailed	LO	6 healthy	10-minute intervals from 7:30 am to 4:30 pm approximately (8.5-hours period reported)
1986	**Farris et al.** [35]	FPD	Clifton Technical Physics	LO	13 healthy	Three measurements during morning visits and three during afternoon visits on the same day*
1986	**Benjamin and Hill** [36]	FPD	No detailed	LO	2 healthy	1-hour interval from 8:00 am to 4:00 pm (six-day period)
1991	**Gilbard et al.**[37]	FPD	No detailed	LO	4 healthy	6:00 am, 9:00 am and 9:00 pm (and the following day at 6:00 am and at 9:00 pm)
2005	**Dalton and Jones**[38]	FPD	Advanced Tear Osmometer	TFO	40 healthy	9:00 am, 12:00 pm and 4:00 pm (two consecutive days)
2011	**Dimit et al.**[39]	EI	TearLab	TFO	- healthy - CLW	1 hour-lens wearing in the morning, and around seven hours later in the afternoon*
2011	**Montani** [40]	EI	TearLab	TFO	50 DED + CLW	Baseline previous CL fitting and after 7 hours of wear*
2012	**Khanal and Millar** [41]	EI	TearLab	TFO	5 healthy	9:00 am,12:00am-1:00 pm and 4:00-4:30 pm (two consecutive days)
2012	**Li et al.** [42]	EI	TearLab	TFO	10 healthy /10 DED	8:30 am,10:30 am, 12:30 pm, 2:30 pm and 4:30 pm
2012	**Oncel et al.** [43]	EI	TearLab	TFO	30 healthy	8:00 am, 11:00 am, 2:00 pm and 5:00 pm
2013	**Niimi et al.** [44]	EI	TearLab	TFO	38 healthy	Upon awakening, 20 minutes, 40 minutes, 1 hour, 2 hours, 4 hours, and 8 hours after awakening*
2013	**Yiğit et al.** [45]	EI	TearLab	TFO	20 healthy / 20 DED	8:00 am, 11:00 am, 2:00 pm and 5:00 pm
2013	**Kim et al.** [46]	FPD	Multi-OSMETTE 2430	LO	10 healthy / 10 medicated	10:00 pm, 12:00 pm, and at 6:00 pm and 10:00 pm the following day
2014	**Montani** [47]	EI	TearLab	TFO	30 CLW	Baseline previous CL fitting, 10 minutes, and 7 hours after fitting*
2014	**Montani** [48]	EI	TearLab	TFO	12 KC + CLW	Baseline previous CL fitting, 20 minutes, and 7 hours after fitting*
2014	**Garcia et al.** [49]	FPD	Fiske 210 osmometer	LO	26 healthy	11:00 am-1:00 pm and 5:00 pm-7:00 pm (three non-consecutive days with 2- to 5-day intervals)
2014	**Lee et al.** [50]	FPD	Multi-OSMETTE 2430	LO	10 healthy /10 SD	2:00 pm and 10:00 pm, and at 6:00 am and 2:00 pm the following day
2015	**Bayhan et al.** [51]	EI	TearLab	TFO	20 healthy / 20 CLW	8:00 am, 10:00 am, 1:00 pm and 4:00 pm
2020	**Pena-Verdeal et al.** [56]	EI	TearLab	TFO	25 healthy	9:30 am and 6:30 pm
2020	**Van Setten** [52]	EI	TearLab	TFO	4 healthy / 4 DED / 2 CLW	8:00-10:00 am, 2:00 pm and 3:00-5:00 pm

Table 2. Mean ± Standard Deviation (range) of tear film osmolality in healthy subjects for the different time-point reported in the literature.
*Exact data for each time-point not available or measurement time not reported.

Year	Authors	Units	Awake			am									pm					
			Immediately	20 min	40 min	6:00	7:30	8:00-8:30	9:00-9:30	10:00	11:00	12:00	1:00	2:00	4:00-4:30	5:00	6:00-6:30	7:00	9:00	10:00
1978	**Terry and Hill** [33]	*mOsm/kg*	285 ±2.4 (282–288)							310 ± 5.7 (299 - 323)*										
1983	**Benjamin And Hill** [34]	*mOsm/kg*					318 ±31 (231-446)*													
1986	**Farris et al.** [35]	*mOsm/l*				306 ±7 (295–316)*									308 ± 8 (297–320)*					
1986	**Benjamin And Hill** [36]	*mOsm/kg*						315–348	343- 337	335-343	325-326	314-321	313-373	307-360	328-334					
1991	**Gilbard et al.**[37]	*mOsm/l*	(296–299)			(296–299)			(300–306)										(300–308)	
2005	**Dalton and Jones**[38]	*mOsm/kg*							306.1 ±11.9 (266–349)*											
2011	**Dimit et al.**[39]	*mOsm/l*				296.90 ±8.51*									298.90 ±8.71*					
2012	**Khanal and Millar** [41]	*mOsm/l*							310.96 (286–356)*											
2012	**Li et al.** [42]	*mOsm/l*						298 ±14.2 (278 – 342)*												
2013	**Niimi et al.** [44]	*mOsm/l*	264 ±14	287 ±10	292 ±16	293 ±12 / 297 ±1*														
2013	**Yiğit et al.** [45]	*mOsm/l*						302.5 ±12.53			301.7 ±9.64			299.85 ±6.98		299.55 ±10.1				
2013	**Kim et al.** [46]	*mOsm/kg*								295.8 ±6.3 299.3 ±4.3		299.2 ±6.0					306.3 ±5.0			
2014	**Garcia et al.** [49]	*mOsm/kg*									270.00 ±4.4*					270 ±4.4*				
2014	**Lee et al.** [50]	*mOsm/kg*		309.2 ±2.4		309.2 ±2.4								303.2 ±4.8 302.9 ±3.1						303.7 ±4.4
2015	**Bayhan et al.** [51]	*mOsm/l*						293.2 ±6.4 294.0 ±6.3		292.3 ±6.3 292.1 ±6.1		290.6 ±5.1 292.4 ±6.7			292.3 ±4.1 293.3 ±4.9					
2020	**Pena-Verdeal et al.** [56]	*mOsm/l*							309.96 ±9.0								296.48 ±12.9			
2020	**Van Setten** [52]	*mOsm/l*						305 (302 - 307)						298 (288 - 303)	317 (304 - 340)					

Table 3. Mean ± Standard Deviation (range) of tear film osmolality in subjects with altered tear film conditions or environment for the different time-point reported in the literature. CLW: Contact Lens Wearer. DED: Dry Eye Disease. KC: Keratoconus. SD: Sleep-Deprived. SiHy: Silicone hydrogel contact lenses. CHy: conventional hydrogel contact lenses. RGP: Rigid gas permeable lens. *Exact data for each time-point not available or measurement time not reported.

Year	Authors	Units	Study group	am						pm									
				6:00	7:30	8:00-8:30	9:00	10:00	11:00	12:.00	1:00	2:00	4:00-4:30	5:00	6:00	7:00	9:00	10:00
2011	**Dimit et al.** [39]	*mOsm/l*	*CLW (SiHy)*	303.32 ±8.41*									300.10 ±10.51*					
2011	**Montani** [40]	*mOsm/l*	*DED + CLW (CHy)*	323 ±4.3*									325 ±13.3*					
			DED + CLW (CHy)	323 ±4*									340 ±15.6*					
2012	**Li et al.** [42]	*mOsm/l*	*DED*	304 ±10.8 (287 – 342)*														
2013	**Yiğit et al.** [45]	*mOsm/l*	*DED*			319.65 ±15.8			314.55 ±13.02			317.95 ±14.72		316.3 ±15.32				
2013	**Kim et al.** [46]	*mOsm/kg*	*Medicated (Ambroxol)*					295.5 ±6.2 298.3 ±6.6		297.3 ±7.0					311.8 ±5.6			
2014	**Montani** [47]	*mOsm/l*	*CLW (SiHy)*	303 ±8.83* - 303 ±18.74*									321 ±23.12*					
2014	**Montani** [48]	*mOsm/l*	*KC + CLW (RGP)*	295 ±10.84* - 281 ±7.07*									298 ±5.86*					
2014	**Lee et al.** [50]	*mOsm/kg*	*SD*	331 ±3.7								300.1 ±3.8 302.2 ±4.5						303.2 ±4
2015	**Bayhan et al.** [51]	*mOsm/l*	*CLW (SiHy)*			292.2 ±5.4 292.9 ±6.1		294.5 ±6.8 294.6 ±5.9		303.4 ±6.6 303.4 ±6.2			294.2 ±6.2 293.9 ±6.3					
2020	**Van Setten** [52]	*mOsm/l*	*DED*			350 (334 - 390)						398 (301 - 354)	327 (303 - 359)					
			CLW			296						304	316					

707 **Table 4.** Summary of the principal finding in tear film osmolarity diurnal variation analysis reported by the literature. CLW: Contact Lens Wearer.
708 DED: Dry Eye Disease.

Year	Authors	Conclusion	Main limitations to the conclusions
1978	**Terry and Hill** [33]	Tear osmolarity was not affected by the time of the day in this normal cohort of patients	No Specific tear film osmometer, small sample analysed
1983	**Benjamin and Hill** [34]	There was an overall trend during the day towards increased hyper tonicity	No Specific tear film osmometer, small sample analysed
1986	**Farris et al.** [35]	There was no significant difference in the means in the healthy group during the morning and afternoon measurements	No Specific tear film osmometer, small sample analysed, wide time range stablished on time-point
1986	**Benjamin and Hill** [36]	There were variation on the osmolarity over the day	No Specific tear film osmometer, small sample analysed
1991	**Gilbard et al.** [37]	Tear osmolarity was not affected by the time of day in this normal cohort of patients	No Specific tear film osmometer, small sample analysed
2005	**Dalton and Jones**[38]	Average tear film osmolality varies little over the day, but individual variations may be significant	-
2011	**Dimit et al.** [39]	There was no statistical significance between morning and afternoon visits for either CLW or non-CLW	Sample not size not right described
2011	**Montani** [40]	Lenses made from different materials can have a different impact on the daily tear film osmolarity	-
2012	**Khanal and Millar** [41]	No diurnal variation was observed in the average readings for tear osmolarity	-
2012	**Li et al.** [42]	Variations in the tear osmolarity of individual DED and healthy subjects were documented during an 8-hour daytime period	Small sample analysed
2012	**Oncel et al.** [43]	In normal subjects, tear osmolarity seems to have a stable profile during the daytime	Small sample analysed
2013	**Niimi et al.** [44]	Diurnal variation on healthy subjects significantly changes upon awakening and remains relatively constant throughout most of the day	Small sample analysed
2013	**Yiğit et al.** [45]	There was no significant change in daytime variations of the tear osmolarity in dry eye patients and healthy subjects	-
2013	**Kim et al.** [46]	Tear osmolality in healthy and medicated groups increased at 6 pm and returned to the baseline value the next morning (although osmolarity in the medicated group was significantly higher than that in the control group)	No Specific tear film osmometer, small sample analysed
2014	**Montani** [47]	After wearing a silicone hydrogel contact lens tear osmolarity increased compared to baseline and measurements done after the fitting.	No control group
2014	**Montani** [48]	In adapted patients to RGP contact lens an initial decrease in tear osmolarity in contact lens insertion followed by a return towards baseline values after wearing	No control group, small sample analysed
2014	**Garcia et al.** [49]	There were no significant differences between different days and the times of day	No Specific tear film osmometer, wide time range stablished on time-point
2014	**Lee et al.** [50]	Sleep deprivation induced tear hyperosmolarity over normal healthy subjects	No Specific tear film assessment osmometer, small sample analysed
2015	**Bayhan et al.** [51]	Tear osmolarity increases within the first hours of CLW, and instillation of osmoprotective eye drops prevents this increment	-
2020	**Pena-Verdeal et al.** [56]	Tear film osmolarity does appear to have some influence by the time of day in healthy patients.	-
2020	**Van Setten** [52]	Daily variation in osmolarity, maximal amplitude of osmotic variation, frequency of osmotic cycles, the level of osmolarity at which the variation does occurs could be the main factors that further characterize osmokinetics.	Small sample analysed; wide time range stablished on time-point

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