Basal Tear Osmolarity as a metric to estimate body hydration and dry eye severity

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35

Table of Contents

36

37

38

39

40

41

42

43

44

45

46

47

Abstract:

The osmolarities of various bodily fluids, including tears, saliva and urine, have been used as indices of plasma osmolality, a measure of body hydration, while tear osmolarity is used routinely in dry eye diagnosis, the degree of tear hyperosmolarity providing an index of disease severity. Systemic dehydration, due to inadequate water intake or excessive water loss is common in the elderly population, has a high morbidity and may cause loss of life. Its diagnosis is often overlooked and there is a need to develop a simple, bedside test to detect dehydration in this population. We hypothesize that, in the absence of tear evaporation and with continued secretion, mixing and drainage of tears, tear osmolarity falls to a basal level that is closer to that of the plasma than that of a tear sample taken in open eye conditions. We term this value the Basal Tear Osmolarity (BTO) and propose that it may be measured in tear samples immediately after a period of evaporative suppression. This value will be particular to an individual and since plasma osmolarity is controlled within narrow limits, it is predicted that it will be stable and have a small variance. It is proposed that the BTO, measured immediately after a defined period of eye closure, can provide a new metric in the diagnosis of systemic dehydration and a yardstick against which to gauge the severity of dry eye disease.

Key Words: tears, plasma osmolarity, osmolality, systemic dehydration, dry eye

1. INTRODUCTION

In this paper we hypothesize that in the healthy eye, tear osmolarity measured after a period of evaporative suppression, represents a basal level of osmolarity close to that of the plasma. It is proposed that such a metric can provide a valuable measure of body hydration and a baseline against which to gauge the severity of dry eye disease (DED).

The aqueous tears occupy the conjunctival sac when the eyes are closed and are redistributed between the fornical and preocular compartments when the eyes open (Gaffney et al., 2010). The preocular compartment splits into two during the upstroke of the blink to form the preocular tear film and the tear menisci, and these are surfaced anteriorly by the tear film lipid layer, which retards evaporation (McDonald and Brubaker, 1971; Peng et al., 2014; Cerretani and Radke, 2014). Once formed, the tear film remains 'perched' throughout the blink interval (Miller, Polse and Radke, 2002) while the menisci provide a conduit for the drainage of tears into the nasolacrimal system (Doane, 1981). The tear film is further divided into the precorneal and prebulbar films.

The aqueous tears derive chiefly as an active secretion of the lacrimal gland (Mircheff, 1989; Turpie et al., 2009; Dartt 2004, 2009; Hodges and Dartt, 2016; Stevenson, Pugazhendhi, and Wang 2016), and to a lesser extent from, the conjunctival epithelium, including the goblet cells (Shiue et al., 2000; Dartt, 2002; Li et al., 2001; Dartt, 2009) and the corneal epithelium (Klyce and Crosson 1985). The size of these additional contributions is not established in humans but in the rabbit it has been calculated that the conjunctival fluid could account for the volume of the basal tear secretion (Shiue et al., 2000; Li et al., 2001). Cerretani and Radke, in their model of human tear dynamics concluded that the contribution of osmotically-induced water flow to the total tear supply, through the conjunctiva and cornea, was in the region of 10% (Cerretani and Radke, 2014). In patients who have undergone daryoadenectomy (removal of the main and palpebral parts of the lacrimal gland) in the treatment of epiphora (Taiara and Smith, 1973; Hornblass, Guberina, and Herschorn, 1988) or lacrimal gland neoplasia (Rose and Wright, 1992), a proportion of patients fail to develop dry eye and may show no reduction in the Schirmer response, implying an adequate supply of tear fluid from some source other than the main and palpebral lacrimal gland (Stevenson Pugazhendhi and Wang, 2016). This source could include the accessory lacrimal glands and

the conjunctival and corneal epithelia but such reports do permit the relative contribution of these sites to residual tear secretion to be determined. The accessory glands account for about a tenth of the total lacrimal mass (Allansmith et al., 1976). Thus they do not shed light on the normal contribution of the ocular surface epithelia to tear production and this fraction remains unknown in humans. The lacrimal component increases substantially during emotional tearing and in the reflex response to intense light or a corneal foreign body (Murube, 2009; Dartt, 2002; Nelson and Wright, 1986).

2. LACRIMAL SECRETION

The acinar cells of the lacrimal gland represent about 80% of the glandular mass while the duct cells represent 10-12% (Dartt, 2002). The *lacrimal secretion*, derived from the lacrimal acini, is modified as it passes through the lacrimal ducts and its composition differs from that of the *lacrimal fluid* that is delivered into the conjunctival sac. Regulated secretion of the major acinar proteins, lysozyme, lactoferrin, lipocalin, and peroxidase, involves exocytosis, a rapid process involving the fusion of acinar apical membranes with those of the apical secretory vesicles, occurring in response to an appropriate stimulus. The duct epithelium modifies the primary lacrimal secretion by the addition of water and electrolytes, particularly of K^+ and Cl^- ions (Dartt, Moller, and Poulsen, 1981; Mircheff, 1989; Ubels et al., 1994; Dartt, 2009; Katona et al., 2014). In the rabbit, it has been estimated that the duct cells secrete about 30% of the lacrimal fluid (Katona et al., 2014) but the figure for human lacrimal fluid is not known.

The lacrimal, conjunctival and corneal fluids are mixed and distributed by blinking (Gaffney et al., 2010) and to a lesser extent by eye movements (Yokoi, Bron and Georgiev, 2014) and it is this composite fluid that is termed the tears and is assayed in meniscus samples.

3. TEAR OSMOLARITY / TEAR OSMOLALITY

The *osmolarity* of a solution is the number of osmoles per litre of solvent, usually expressed as milliosmoles. The *osmolality* of a solution is the number of osmoles per kilogram of solution. In the literature related to systemic disease and plasma, osmolality is the preferred term while in the tear literature the term osmolarity is more often used. Where an estimate for serum is made from the concentration of selected serum constituents, the value is usually

expressed as osmolarity. Clinically the numerical difference between the two terms may be small and but the formula selected to make the calculation is of importance (Hooper, 2015a). Here we use either term, according to its literature source.

For the tears, based on a meta-analysis of several studies using depression of freezing point, or vapour pressure measurement, tear osmolarity (tOsm) has been reported to be 302 ± 9.7 mOsm/L in normal adults (Tomlinson et al., 2006). Similar values were reported by Sullivan et al. (Sullivan et al., 2010) - 302.2 ± 8.3 (n = 75), Jacobi et al. (Jacobi et al., 2011) - 301 mOsm/L (n=95), Keech et al. (Keech et al., 2013) - 301.2 ± 7.2 mOsm/L (n=15), Eldridge, et al (Eldridge et al., 2010) - 301.8±10.5 mOsms/L and by Li et al. (Li et al., 2012) - 298.0±14.2 mOsms/L, based on smaller subsets. In all these studies, tear osmolarity values were obtained using the TearLab® device, which depends on the measurement of electrical impedance and therefore the presence of charged particles in solution and therefore, in the tears, mainly ions and to a much lesser extent, proteins. The presence of urea and of glucose in the tears is not registered by this device.

Tear hyperosmolarity is the central mechanism of dry eye acting in part directly on epithelial cells (Kam et al., 2016) and in part by generating downstream inflammatory events at the ocular surface (Bron et al., 2017). Tear film break-up in the blink interval amplifies tear hyperosmolarity and additionally, degrades optical performance when tear instability and breakup intrude upon the visual axis (Chao et al., 2016).

When the eyes are open, the osmolarity of the tears is modified by evaporation, to an extent that depends on ambient humidity (Madden, Tomlinson and Simmons, 2013; Lee et al., 1999), air temperature (Abusharha Pearce and Fagehi, 2015) and airflow (Peng et al., 2014), the size of the palpebral aperture and the length of the blink interval, which determines the period of evaporation (Tsubota and Nakamori, 1995; Tsubota, 1998). Tear osmolarity is increased by a low relative humidity (RH), high wind speed, raised air temperature, a wide palpebral aperture and an extended blink interval (Chao et al., 2016). It is generally stated that the lacrimal fluid is secreted as an iso-osmotic, or slightly hypo-osmotic fluid (Terry and Hill 1978; Gilbard and Farris, 1979; Niimi et al., 2013) compared with plasma. Tears

sampled from the menisci are considered to have a higher level of osmolarity than that of secreted tears, (Mishima and Maurice, 1961; Mishima, 1965; Niimi et al., 2013; Cerretani and Radke, 2014) and that of the tear film, the latter due to the differential effect of evaporation on these two compartments during the blink interval (Gaffney et al., 2010). The ionic composition of the tears is determined by the secretory process (Dartt, 2009; Mircheff, 1989; Katona et al., 2014) and it has been noted that the relative proportions of electrolytes measured in tear fluid and plasma differ (van Haeringen, 1981). Thus, while the concentrations of Na^+ and $HCO3^-$ in human tears are close to those of the plasma (Krogh Lund and Pedersen-Bjergaard, 1945; Hind and Goyan, 1949; Thaysen and Thorn, 1954; Yoshimura and Hosokawa, 1963), those of $K^+$and Cl^- are higher in the tears (Rismondo et al., 1989), and there is evidence in the rabbit (Mircheff, 1989; Ubels et al.,1994) and rat (Ubels et al., 2006) that K^+ and Cl^- ions are added to the lacrimal fluid by the epithelial cells of the lacrimal duct. In a carefully designed study in rabbits, reported by Yoshimura and Hosokawa (Yoshimura and Hosokawa, 1963) in which tear osmolarity was measured by freezing point depression, tear osmolarity was 17mOsm/L higher in the tears than in plasma (329 in tears versus 312 mOsm/L in plasma) due to the higher K^+ and Cl^- ion concentrations in the tears. In other reports, also in the rabbit, lacrimal fluid osmolarity was reported to be inversely proportional to flow rate, with hyperosmolarity encountered at low secretory rates (Bothelo and Martinez, 1973; Gilbard and Dartt, 1982). Botelho and Martinez postulated that at low flow rates, water might be reabsorbed in the ducts, distal to the acini. If this situation applies to human tears then it cannot be excluded that human lacrimal fluid too, is slightly hyperosmolar with respect to plasma.

3.1. Diurnal Variation of Tear Osmolarity

Various researchers have reported a diurnal variation of tOsm, with the tears found to be hypo-osmotic on waking (Terry and Hill, 1978; Niimi et al., 2013). Niimi et al. (Niimi, et al., 2013) used a TearLab® apparatus modified to register lower levels of osmolarity, to study the relationship between diurnal variations of tear osmolarity, central corneal thickness and corneal deswelling over the day. The TearLab® device measures tear osmolarity on the basis of electrical impedance and has the advantage that measurement is made directly on the sampled fluid, without risk of evaporative loss. The authors recorded osmolarity at bedtime (base-line), upon waking after 6-8.5 hours sleep and at intervals after waking. Tears on waking were found to be significantly hypo-osmotic (264 ± 14 mOsms/L) compared with the pre-sleep, baseline values of 297 ± 15 mOsms/L and those encountered later in the day. Tear osmolarity rose quickly in the first 10 minutes after waking, reaching baseline levels within the first 40 minutes (P = 0.085). These authors attributed the hypo-osmolarity of tears on waking to the suppression of evaporation by lid closure and possibly to reflex tearing occurring on eye opening. Also, their subjects were instructed to blink 3 times and to squeeze their eyes shut to release fresh tears prior to tear collection, and this may have influenced the outcome. Given that the level of osmolarity reported fell below that normally cited for plasma osmolarity, (i.e. 285-295 mOm/kg (Matz, 1996; Stookey, 2005; Cheuvront et al., 2010) reflex tearing at the time of sampling may have contributed to the low value, but does not explain it.

In conditions of high tear flow, such as with reflex tearing, tOsm falls from that recorded in unstimulated, open eye conditions. In a study of six subjects with normal eyes, exposed to the beam of the slit lamp for five seconds, to induce reflex tearing, tOsm measured by a depression of freezing point method, fell from 303.2 ±7.2 mOsm/kg (range 287-312 mOsm/kg), to 289.5 ±6.8mos/kg (range 275-298 mos/kg), a 5% decrease, which was statistically significant ($p<.001$) (Nelson and Wright, 1986).

4. DRY EYE DISEASE

Dry eye disease is a symptomatic eye disorder in which drying of the exposed ocular surface by evaporative water loss, results in tear hyperosmolarity. This damages the ocular surface, either directly or by a chain of events causing inflammatory ocular surface damage. (Bron et al., 2017) There are two major subtypes. In aqueous-deficient dry eye (ADDE), tear hyperosmolarity is due to evaporation from a reduced tear flow, caused by a reduction in lacrimal secretion. In evaporative dry eye (EDE), tear hyperosmolarity arises from an

excessive evaporative loss, caused by a failure of the barrier function of the tear film lipid layer and amplified by tear film break up. Tear hyperosmolarity has been proposed as the best single diagnostic test of dry eye (Korb, 2000). In a multicentre study the most sensitive threshold distinguishing normal from mild/moderate dry eye disease was 308 mOsm/L and the most specific cut off was 315 mOsm/L (Lemp et al., 2011). In terms of tear osmolarity, severity is compared with values in subjects with normal eyes. The hypothesis that we present below gives the opportunity to use a tOsm value obtained in the same individual, rather than derived from a normal, control population.

5. BODY HYDRATION and DEHYDRATION

Total body water (TBW) makes up about 50%–60% of the body mass, with about two thirds being intracellular, predominantly in lean tissue, and the remainder extracellular (Danziger and Zeidel, 2015). Blood contributes about 8% to the TBW (Rikkert, 1998; Bossingham, et al., 2005). Water is lost from the body as insensible perspiration and sweat and in respiratory vapour, urine and faeces and is replaced by fluid intake and by water contained in foodstuffs. At sea level, the amount of water lost as respiratory vapour is balanced by metabolic water production (Cheuvront et al., 2014).

Regulation of water balance is fundamental to survival and is achieved by a combination of water conservation (renal) and acquisition (thirst). Water conservation results from the action of arginine vasopressin (AVP or antidiuretic hormone) on renal water absorption (Baron, 2015). AVP is synthesised in the supraoptic and paraventricular nuclei of the hypothalamus and delivered to the posterior pituitary, from which it is released (Bourque, 2008) in response to signals from hypothalamic osmoreceptors (eg.TRPV1) (Ciura, 2006; Leng, 1982). These, acting as membrane stretch-receptors, signal changes in cell volume (Liedtke, 2000) in response to changes in plasma osmolality (pOsm). A rise in pOsm creates an osmotic gradient through which the effects of water loss are shared between the intracellular fluid (ICF) and extracellular fluid (ECF) compartments (Cheuvront and Kenefick, 2014). An increase in neuronal firing stimulates the release of AVP from the posterior pituitary, resulting in renal water reabsorption, urinary concentration and water conservation (Cheuvront et al., 2013). A rise is pOsm also stimulates an increase in water intake in response to thirst (Egan, et al., 2003) which is independent of the action of AVP and results from direct neural signaling (Denton, et al., 1999; Bourque, 2008). Peripheral osmoreceptors,

eg. in the gut, also play a role (Bourque, 2008).

The osmoreceptor neurons in the hypothalamus are believed to encode an osmotic set-point (Bourque, 2008) that keeps pOsm from deviating by more than 1-2% in an individual (Bourque, 2008; Cheuvront and Kenefick, 2014). The pOsm set point for AVP release is lower than that which stimulates thirst by 10 mmol/kg or more (Cheuvront, et al., 2013).

In this way, in normally hydrated subjects, hydration is maintained within narrow limits. (Danziger and Zeidel, 2015). For plasma, this is between 285-295 mOsm/kg. (Matz, 1996; Stookey, 2005; Cheuvront, 2010). Thomas et al. cite a broader range for serum osmolality of 275 to < 295 mOsmol/kg, (Thomas et al., 2008) but < 2% of free-living people have a pOsm <285 mOsmol/kg when they consume ≥ 3.0 L fluid per day (Stookey, 2005).

Clinical dehydration has been defined as a loss of body water, with or without salt, at a rate greater than the body can replace it (Thomas et al., 2008). This article is concerned with the water-loss dehydration, which is accompanied by intracellular dehydration, plasma hyperosmolarity and, usually, plasma hypernatraemia. It is also termed hypohydration, hyperosmotic hypovolaemia and dehydration with minimal salt loss (Cheuvront and Kenefick, 2014). Water-loss dehydration may also be due to hyperglycaemia, in which case it is accompanied by hyponatraemia. Extracellular dehydration, caused by a loss of iso-osmotic body fluids, as in secretory diarrhea, involves a reduction in ECF water and will not be discussed here. (Cheuvront and Kenefick, 2014),

Plasma or serum osmolality, measured directly, or estimated from the chemical composition of these fluids (Hooper, 2015a; 2016) has long been used as a clinical index of body hydration (Armstrong, 2007; Cheuvront et al., 2010; Baron et al., 2015) serving as the gold standard against which other less invasive methods are compared in the diagnosis of dehydration. Clinical or 'current' dehydration is defined by a plasma osmolality of > 300 mOsm/kg and preclinical, or 'impending' dehydration by a plasma osmolality of > 295 and ≤ 300 mOsm/kg. Impending dehydration can be managed by a planned adjustment of an individual's daily fluid intake, whereas current dehydration demands urgent water replacement to prevent life-threatening complications. Loss of body mass ≥3%, signifying loss of TBW, recorded over a period of 7 days, is also used as a reference standard in the detection of dehydration, (Hooper et al., 2016).

The frequency of current dehydration in the elderly population is high, with impending dehydration reported as 40% in those aged 70-90 years, in the US NHANES III cohort, with a further 28% exhibiting current dehydration (referred to in this report as, 'overt hypertonicity' , ≥ 300mmol/L) (Stookey, 2005). Consequently, dehydration, contributing to the risk of chronic diseases such as urolithiasis, hypertension and coronary heart disease, (Xiao, Barber, and Campbell, 2004), is a leading cause of hospitalization and death in the elderly. (Manz and Wentz, 2005; Oei et al., 2016) A number of factors contribute to this. Older people have a smaller body fluid reserve than younger people, due to reduced muscle volume (Rickert et al.,1997; Martin et al., 1994) and lose more intracellular water and less interstitial fluid in response to heat and exercise (Morgan, et al., 2002). Food intake and the number of episodes of drinking decrease with age (Gaspar, 1999) and the elderly fail to drink adequate amounts of fluid in response to dehydration (Rolls and Phillips, 1990) in part due to a decreased sense of thirst (de Castro, 1992). The urinary concentrating ability of the kidney also declines with age (Davies et al., 1995; Lindeman et al., 1985; Morely, 2000; Sands, 2012; Hooper et al., 2016) and, additionally, an increased use of diuretics or laxatives in older people contributes to greater fluid loss (Mentes, 2006). Other, cognitive and physical factors, reduce fluid intake (Lindeman et al., 2000; Zizza et al., 2009) and drinking may be restricted deliberately as a measure to control incontinence (Hooper et al., 2016; He et al., 2015). Those with dementia may forget to drink, as daily routines are lost and social contacts diminish (Hooper et.al., 2016).

The risk of dehydration is increased in elderly patients in long-term care. Hooper et al. (Hooper et al., 2016) reported a frequency of 20% in a population of care home residents (n=188) with a mean age 86 years, with renal, cognitive and diabetic status consistently associated with the risk of dehydration. Wolff et al. (Wolff et al., 2015) in another UK study, basing the diagnosis of dehydration on the presence of hypernatraemia on admission to hospital (plasma Na > 145 mmol/L), found a 5-fold increase in the occurrence of dehydration in patients admitted to hospital from care homes (adjusted odds ratio [AOR]: 5.32, 95% CI: 3.85-7.37), compared to that in patients admitted from home, and roughly a two-fold greater risk of in-hospital death (AOR: 1.97, 95% CI: 1.59-2.45) (Wolff et al., 2015).

This background emphasizes the need to detect dehydration in the elderly, both in the wider community and in individuals in care (Hydration for Health Initiative, 2012). Dehydration is

less likely to be overlooked in the hospital population, where serum osmolarity can be readily calculated from blood samples. While it is generally agreed that the estimation of plasma osmolality or serum osmolarity, provide the best single assessment of body hydration (Hooper et al., 2016; Thomas et al., 2008) such tests are not routinely performed in the community or in primary or residential care settings (Leibovitz, 2007). Assessment by health or social care workers is more likely to be based on the demonstration of reduced thirst, sense of a dry mouth, furrowing of the tongue, loss of skin turgor, a dry axilla, slow capillary refilling after compression of the nailbed, and increase in urine colour, which appear to be poor indicators of dehydration in older adults (Hooper et al., 2016). More formal measurements, of urinary specific gravity, or of salivary or urinary osmolarity, or bioimpedance have also been used. In a systematic review of tests validated to detect current water-loss dehydration in older people, Hooper et al (Hooper et al., 2015b) found that only three stand-alone tests showed any ability to diagnose water-loss dehydration, as indicated by a serum osmolality ≥ 295 mOsm/kg, with a sensitivity ≥ 0.60 and specificity ≥ 0.75. These were, missing drinks between meals, expressing fatigue and, in some reports, bioimpedance (BIA) at 50 kHz. No tests were clearly useful in diagnosing current water-loss dehydration (serum osmolality > 300 mOsm/kg).

This report (Hooper et al., 2015b) and that of the earlier, US Panel on Dietary Reference Intakes, (Panel on Dietary Reference Intakes, 2004) emphasize the need to develop a valid, simple and non-invasive screening test of dehydration in the community, to enable the identification and management of water loss dehydration in older adults.

5.1. Body Hydration and Tear Osmolarity.

Although lacrimal secretion is influenced by vascular filtration pressure (Botelho et al., 1976) it is the active, energy-requiring, secretory process that determines the final composition of the tears and hence its osmolarity (Dartt Moller and Poulsen, 1981; Mircheff, 1989). Tear osmolarity is also influenced by plasma osmolarity and the extent to which this occurs in humans has been demonstrated by Walsh and colleagues (Fortes et al., 2011; Walsh Fortes, and Esmaeelpour 2011; Walsh et al., 2012) who reported a positive relationship between whole body hydration measured as pOsm, and tOsm, in subjects exposed to systemic dehydration (Fortes et al., 2011). In a study conducted in an environmental chamber, a group of young adults in their 20s, was exposed to systemic dehydration, equivalent to 2 to 3% loss of body mass, generated by a combination of water-deprivation and a period of physical

exercise. Tear osmolarity followed pOsm closely during the evolution of dehydration and, like pOsm, was restored to normal during rehydration. In this study, the pre-exercise pOsm was 288 ± 5 mOsm/kg. In two trials, the mean tOsm correlated strongly with mean pOsm at each time point ($r = 0.93$, $P < 0.001$), suggesting that tOsm could serve as a minimally invasive surrogate for body hydration. Fortes et al. reported a sensitivity of 80 % and specificity of 92% using tOsm to detect systemic dehydration (Fortes et al., 2011). In a subsequent study, the authors reported that pOsm may be raised in patients with dry eye disease with the implication that the raised tOsm could be a consequence of body dehydration (Walsh Fortes and Esmaeelpour, 2011). In a subsequent letter they expressed the view that this could lead to a misdiagnosis of dry eye in patients who suffered from systemic dehydration, (Walsh et al., 2012) but Tomlinson et al. (Tomlinson Madden and Pearce, 2011) in response, pointed out that the persistent presence of a tear hyperosmolarity within the range consistent with the diagnosis of DED, in conjunction with supportive clinical features, would imply the actual presence of DED. Importantly, as noted by Walsh et al. (Walsh Fortes and Esmaeelpour, 2011), since the risk of both dry eye (Uchino et al., 2006; Moss Klein and Klein, 2008; Guo et al., 2010) and systemic dehydration (Cheuvront and Kenefick, 2014), increases with age, the value of a raised tOsm in the diagnosis of systemic dehydration is the elderly will be reduced (Walsh Fortes and Esmaeelpour, 2011; Walsh et al., 2012; Tomlinson Madden and Pearce, 2011).

It is evident that the occurrence of tear hyperosmolarity due to DED is a potential source of false positives when using tear osmolarity to diagnose systemic dehydration, when based on the results of random, open eye tear samples. However, if, as we propose below, the tOsm measurement were to be made after a period of evaporative suppression, this difficulty would be overcome and a realistic estimate of both body hydration status and of dry eye severity could be achieved

6. HYPOTHESIS

6.1 Basal Tear Osmolarity as a Metric in Dry Eye Diagnosis and in the Estimation of Body Hydration

As noted, tear hyperosmolarity is the central mechanism in dry eye disease. At present, for diagnostic purposes, when a patient is suspected of having dry eyes, their tear osmolarity, derived from a meniscus sample, is compared with population norms obtained from subjects

over a wide age range. It would be more valuable if a comparison could be made with that individual's own tear osmolarity obtained before the onset of dry eye. It is our contention that this value can be obtained in any subject, regardless of the presence or absence of dry eye, simply by subjecting the subject to a period of evaporative suppression prior to tear sampling.

We hypothesize that, in the absence of tear evaporation, with continued lacrimal and conjunctival secretion and adequate tear mixing and drainage, the osmolarity of the tears, equilibrating with the interstitial fluid across the ocular surface epithelia, will fall to a basal levels close to that of the plasma. We consider that this basal value will serve both as a measure of body hydration and as a stable baseline against which to compare the tear hyperosmolarity in dry eye. Tear evaporation is readily prevented by eye closure and we postulate that eye closure for a suitable length of time will drive down tear osmolarity to this basal level, regardless of the osmolar starting point in open eye conditions and therefore regardless of the presence of dry eye. This new metric, which we term the *Basal Tear Osmolarity* (BTO) will be particular to an individual and is predicted to have a smaller variance than tear meniscus values measured in non-standardised, open eye, conditions and to be relatively uninfluenced by ambient environmental conditions. It is proposed as a potential tool in the diagnosis of systemic dehydration and as a yardstick against which to gauge the severity of dry eye disease.

We propose here that the BTO can obtained by measuring tear osmolarity after a period of eye closure or exposure of the subject to a humid environment in open eye conditions. These approaches are described briefly here:

7. MEASUREMENT OF TEAR OSMOLARITY AFTER EYE CLOSURE

In order to explore the effect of lid closure on tear osmolarity it is necessary to estimate the likely period of lid closure required to drive down tear osmolarity to a stable, BTO value. Certain considerations need to be addressed. The hypothesis assumes that with the eyes closed for a suitable period of time, the body of tear fluid contained within the conjunctival sac will be completely replaced by lacrimal fluid, supplemented by a smaller amount of fluid of conjunctival origin and less still of corneal origin. The completeness of this process will depend on tear turnover, mixing and drainage. In the absence of blinking, a deficiency of tear mixing during eye closure might be overcome to a limited extent by performing periodic eye

movements. In patients with ADDE, lacrimal secretion and turnover is, by definition, reduced and it would be predicted that in such individuals, the reduction in tear turnover might increase the time required to drive down tOsm to the BTO value. However, the longer the period of eye closure, the greater the opportunity, for tear fluid in the conjunctival sac to equilibrate with the plasma across the conjunctival epithelium and extracellular space. This equilibration can be predicted to be faster in dry eye disease, since epithelial permeability is increased (Yokoi et al., 1997).

7.1. Estimating the Necessary Period of Eye Closure

On eyelid closure, the elevated tear osmolarity inherited from the open eye will be reduced by cessation of evaporation, by tear turnover and by equilibration across the conjunctival epithelium. The time scale of the former is readily estimated. If the total tear turnover rate is 16% per minute, (Tomlinson Doane and McFadyen, 2009) then the flush-out time is approximately 100/16 min = 6.25 min.

Across the surface epithelium, the osmolarity of the tears would lie somewhere between that of the lacrimal fluid and the epithelial fluids. Here, we make a rough estimate of the time taken for the osmolarity of the lacrimal fluid to approximate to that of the conjunctival fluid, considering equilibration across the vascular conjunctiva alone, since the surface area of the human conjunctiva is an order of magnitude greater than that of the cornea (Watsky Jablonski and Edelhauser, 1988).

Some idea of the equilibration rate can be approximated from the short circuit current across the epithelium. Using rabbit data, based on the unilateral removal of chloride from either side of a rabbit conjunctival preparation, the change in the short circuit current is on the scale of $3\mu Acm^{-2}$ (Kompella Kim and Lee, 1993). This can be converted into an equilibration rate across the conjunctiva, first dividing by Faraday's constant, F, to rewrite the short circuit current in terms of ionic flux. Multiplying by conjunctival surface area (human: A_c=18cm^2) (Watsky Jablonski and Edelhauser 1988) converts this flux into a rate of change of total amount of ion. Dividing by tear volume (V=7µl) (Tomlinson Doane and McFadyen, 2009), gives the rate of change of concentration. Finally, dividing by a representative, initial concentration difference of chloride across the epithelium in these experiments, taking the value to be around c*=100 mM/l this entails an equilibration rate of

$$k = 3\mu Acm^{-2} \,.\, A_c/[FVc^*] = 1.2e\text{-}3s^{-1} \,.$$

The associated equilibration timescale is given by $1/k \approx 830s \approx 14mins$. One must accept the caveat that this is a rough approximation.

In summary, the timescales of the system are such that there will be a relatively rapid wash out of the combined fluids over about 6-7 minutes in the normal eye. If lacrimal fluid hyperosmolarity were to be present, a further equilibration across the conjunctival epithelium will be active on a timescale of, very roughly, 14 minutes. The period of 45 minutes of eye closure adopted in the experiments described below, should therefore be adequate to achieve equilibration. Given its limited surface area, the impact of the less permeable cornea is anticipated to be sub-dominant. These estimates would be modified by variations in tear flow rate and the increase in epithelial permeability encountered in dry eye disease. For comparison, Zhu and Chauhan in a model simulation to determine the impact of moisture chambers on dry eye sufferers, explored the effect of raising the evaporation rate to four times the normal rate and then reducing it back to normal (Zhu and Chauhan, 2007). On the basis of this they predicted a restoration of tear osmolarity to baseline values in about 13 minutes.

7.2. Measurement of Tear Osmolarity in Open Eye Conditions in High Ambient Humidity

Exposure of a subject whose eyes are open, to an ambient RH of 100% will also result in a complete suppression of tear evaporation and offers an alternative approach to the estimation of the BTO. Although the value obtained with either approach should be similar there is a practical value in adopting lid closure for clinical purposes, since it does not require a controlled environment chamber or goggles constructed to create a humid environment.

However, exposure to a humid environment offers experimental advantages in tracking the downward path of tear osmolarity over time, since meniscus sampling can be conducted at any point throughout the exposure period. Similarly, this open eye approach offers the opportunity to study osmolar recovery on transfer to a non-humid environment. In the study

of Niimi et al. (Niimi et al., 2013) tOsm rose quickly over the first 10 minutes after waking, reaching baseline levels within the first 40 minutes. In high humidity studies it is likely that the fall in osmolarity towards the BTO will be faster than in closed eye conditions, because mixing and drainage will be facilitated by spontaneous blinking, whereas in the closed eye state, mixing will be more restricted.

8. PILOTING THE EFFECTS OF EYE CLOSURE AND EXPOSURE TO HIGH HUMIDITY ON TEAR OSMOLARITY

We have performed a preliminary study to estimate the BTO in eight normal subjects and eight dry eye patients, after periods of evaporative suppression achieved by either eye closure or exposure to high relative humidity (Willshire et al., 2017). In the eye closure studies, closure was maintained for a period of 45 minutes, and eye movements were performed from time to time to achieve some degree of tear mixing. In a separate study, subjects were exposed to an atmosphere of 70% RH and tOsm was measured in both eyes, every 15 minutes, for a period 45 minutes. Studies were preceded by measurement of tOsm outside the controlled environment chamber (CEC), in uncontrolled, clinic conditions, to provide baseline values. Tear osmolarity was significantly reduced after eye closure, in the right and left eyes analysed independently, in both normal subjects and dry eye patients, to levels in the range accepted for plasma osmolality, i.e. between 285-295 mOsm/L. The average tOsm measured in the left eye of 8 normal subjects, prior to eye closure, was 293.1 ± 5.54 mOsm/L and was 285.9 ± 5.54 mOsm/L ($p = 0.006$) immediately after eye opening. Corresponding values in 8 patients with mild DED, were 302.3 ± 12.4 mOsm/L in the clinic, falling to 286.1 ± 6.60 mOsm/L following eye closure ($p = 0.01$) (Figure 1). Similar results, also statistically significant, were demonstrated in the right eye (Willshire et al., 2017). When these subjects were exposed to 70% RH, which was not expected to suppress evaporation completely, a significant fall in tOsm occurred in one eye only in the normal group, but not in the dry eye group.

9. PREDICTED UTILITY OF THE BTO IN ESTIMATING BODY HYDRATION

Our hypothesis predicts that total evaporative suppression will drive down tear osmolarity to the BTO in both normal subjects and in patients with DED. The BTO value obtained will be

dependent on that individual's hydration state and as noted, would be expected to be confined within narrow limits, reflecting the tight control of plasma osmolarity. This gets over the difficulty that a raised tOsm measured in open eye conditions cannot distinguish the effect of suboptimal body hydration from that of DED (Walsh Fortes and Esmaeelpour 2011; Walsh et al., 2012) and eliminate concerns that environmental factors such as desiccation, sun, wind or rain and behavioural factors such as outdoor exercise, (causing movement convection), that might act as measurement confounders, limiting the application of this approach within sports, wilderness and military medicine (Sollanek et al., 2012; Cheuvront and Kenefick, 2014).

In normal subjects the difference between the BTO, measured as proposed here and a random meniscus reading measured in clinic conditions, may be predicted to be small, however, in DED, the difference should rise progressively with increasing disease severity. We propose that this differential will provide a better index of dry eye severity in an individual patient than would be afforded by a comparison with a control population.

10. SUMMARY AND CONCLUSIONS

The BTO is proposed here as a new metric for the diagnosis of systemic dehydration and as a yardstick against which to gauge the severity of dry eye disease. This could meet the need expressed by several authors for a technology that is simple, rapid and non-invasive (Armstrong, 2005; Institute of Medicine, 2005; Sollanek, et al., 2012; Ungaro et al., 2015; Holland et al., 2017). Such a metric could be of utility in several ways.

1. It is anticipated that the BTO will provide a better diagnostic surrogate for whole body (plasma) hydration than tear osmolarity measured under non-standardised ambient conditions. As a minimally invasive, point-of-care diagnostic test that can be deployed at the bedside, it may be of value in the diagnosis of dehydration in the elderly. However, if it transpires from future studies, that the BTO can be acquired after a short period of eye closure, say 15 minutes or less, regardless of the starting level of tOsm, then the utility of the test will be greatly enhanced and it may be of value in other situations where individuals are exposed to excessive water loss or deprivation, as in sports and the military environment. Ungaro et al. (2015) compared mean tOsm (averaged between right and left eyes) with pOsm in a group of male athletes, before and after exercise tasks conducted on a stationary cycle

ergometer. These were carried out under controlled environmental conditions, with or without water restriction leading to up to 3% of body mass loss, and also after rehydration. They found that tOsm tracked group changes in hydration status similarly to pOsm but that individual responses of tOsm were less predictable. They concluded that tOsm is a valid indicator of hydration status at the group level, but that large differences among subjects in the response of tOsm to changes in hydration status limited its validity at the individual level (Ungaro et al., 2015). A similar conclusion was drawn in another study conducted under field conditions involving a self-paced 10 km run, in which participants were exposed to varied conditions of temperature, humidity and wind speed (Holland et al., 2017). In that study, although significant reductions in body mass and increases in plasma osmolality, tear osmolarity and urine specific gravity were observed, the pre- to post-exercise change in tear osmolarity was not significantly correlated with plasma osmolality, relative body mass loss, or urine osmolality or specific gravity. It may be surmised that exclusion of environmental exposure, as proposed for a closed-eye BTO test, might have revealed a correlation between tOsm and pOsm in such studies. Importantly, since sampling is performed immediately after a period of eye closure, it will not be influenced by ambient environment or the presence of dry eye disease; the tOsm will be driven down to the BTO level in *any* individual. The time taken to achieve the BTO value in a closed eye test will be important in determining its practicality, particularly under field conditions.

2. It is proposed that measurement of the BTO will be of value in assessing the severity of dry eye, since it will indicate how far tear osmolarity has risen above the basal level in that individual. The set point of pOsm about which pOsm oscillates during the maintainance of osmolar hydration differs between individuals and the threshold and slope (sensitivity) of the AVP response to pOsm change, is under genetic control (Zerbe et al., 1999; Cheuvront et al., 2013). Also, in treating patients with dry eye and trying to restore a normal tear osmolarity, the BTO will provide an appropriate reference point against which to judge successful treatment. Experimentally, the approach also offers the opportunity to explore the time taken for tear osmolarity to return to DED levels on eye opening, in defined ambient conditions. This has some bearing on the recuperative value afforded by eye closure during sleep and may differ between the main subtypes of DED.

3. While the difference between the BTO and the level of tear hypermolarity are conceived to be a measure of dry eye severity, it also indicates the fold increase in tear osmolarity due to

evaporative water loss and therefore how much of the increased concentration of a given solute reflects the concentrating effect of evaporation and how much is due to increased expression of that molecule. It is important to know this, since the level of tear lacrimal protein falls in ADDE but is predicted not to do so in EDE, where lacrimal function is normal (Bron et al., 2009).

4. The role of tear evaporation in causing DED has long been recognized (Lemp, 1995; DEWS 2007; DEWS II - Bron et al., 2017) and treatment measures designed to reduce evaporative water loss are part of the therapeutic approach to dry eye disease, either by the provision of moisture-conserving spectacles (Tsubota Yamada and Urayama, 1994) or, in severe DED, by performing tarsorrhaphy, as a temporary measure (Welch and Baum, 1988; Nelson, 1989; Valim et al., 2015). It is self-evident but rarely emphasized, however, that while in the dry eye patient, overnight eye closure during sleep removes the physical basis of hyperosmolarity, interactions with the proinflammatory conditions induced by the closed eye state (Sack et al., 2000) make its therapeutic implications difficult to predict. It is not expected that the effect of eye closure on tOsm and the level of inflammatory mediators in the tears and tissues will be concordant. We predict that while tOsm will fall, the level of inflammatory mediators will not be affected in the short term and might even increase.

5. In the diagnosis of DED it is recommended that tOsm is measured in both eyes, since the between-eye difference increases in DED and is of diagnostic value. In the report of Lemp et al. (Lemp et al., 2011) normal subjects demonstrated a mean inter-eye difference of 6.9 ± 5.9 mOsms/L, whereas patients with mild or moderate DED demonstrated a difference of 11.7 ± 10.9 mOsms/L and those with severe DED, a difference of 26.5 ± 22.7 mOsms/L. It is likely that, for the detection of systemic dehydration, it will be sufficient to take the measurement in one eye only, after bilateral eye closure, although this will need confirmation based on comparative studies.

6. It has been argued that plasma osmolarity may be of less value in the diagnosis of chronic dehydration than acute dehydration (Armstrong, 2007; Baron et al., 2015), for instance because dehydration may cause a rise in plasma osmolarity that still falls within the normal range and yet represents dehydration in that individual. However, in the environment of a care home for the elderly it would be possible to obtain baseline BTO readings when the patient was in a state of euhydration, against which to compare subsequent measurements.

7. In summary, measurement of the Basal Tear Osmolarity is proposed as a new diagnostic approach worthy of further consideration. Its utility in the diagnosis of body dehydration in the elderly could usefully be studied in the environment of the nursing home and compared to that achieved using current practices. Preliminary studies suggest that, as predicted, the variance of BTO measurements in both normal and DED subjects, is lower than that of the tOsm measured in uncontrolled, clinic conditions, (Willshire et al., 2017). Future studies are planned in larger populations and will include a direct comparison of the BTO with pOsm at different levels of body hydration and the measurement of the BTO in open eye conditions at an RH close to 100%. By conducting such studies in patients with different subtypes of DED, we hope to better define the period of eye closure required for a substantive, clinical BTO test.

Although current studies have indicated, in a preliminary way, a numerical similarity between BTO values measured by the TearLab® device and reference values for pOsm in normally hydrated individuals, it must be recognised that osmolarity measured by electrical impedance does not fully represent the concentration of all particles in solution and hence must be expected to slightly underestimate the full osmolarity of the tears.
The TearLab® device detects the presence of charged particles, such as ions and does not recognize uncharged molecules such as urea or glucose. Urea is a permeant molecule whose concentration in the tears is similar to that in the plasma, accounting, according to one source, for around 6 mOsm/L in normal subjects (Gavrilov et al., 2000). Tear glucose, in non-diabetic subjects contributes about 0.2 mOsm/L (Sen and Sarin, 1980). Thus it may be predicted that when direct comparisons of tOsm and pOsm are made, the pOsm will be about 6 mOsm/L higher than the simultaneously measured tOsm. This prediction needs to be confirmed by a direct comparison of tOsm with pOsm in the same individuals combined with measurements of plasma composition, but does not diminish the potential values of the proposed approach.

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