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Original Communication

Oral antioxidant therapy for marginal dry eye

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Objective: To assess the efficacy of an orally administered antioxidant dietary supplement for managing marginal dry eye.
Design: A prospective, randomised, placebo controlled trial with cross-over.
Setting: Eye Clinic, Department of Vision Sciences, Glasgow Caledonian University.
Subjects: Forty marginal dry eye sufferers composed of 30 females and 10 males (median age 53 y; range 38–69 y).
Interventions: Baseline assessments were made of tear volume sufficiency (thread test), tear quality (stability), ocular surface status (conjunctival impression cytology) and dry eye symptoms (questionnaire). Each subject was administered courses of active treatment, placebo and no treatment, in random order for 1 month each and results compared to baseline.
Results: Tear stability and ocular surface status were significantly improved following active treatment ($P < 0.05$). No changes from baseline were detected following administration of placebo and no treatment ($P > 0.05$). Absolute increase in tear stability correlated with absolute change in goblet cell population density. Tear volume was not improved following any treatment period and dry eye symptom responses were subject to placebo effect.
Conclusions: Oral antioxidants improved both tear stability and conjunctival health, although it is not yet understood whether increased ocular surface health mediates increased tear stability or vice versa.
Sponsors: This study was supported by a PhD scholarship funded by the Department of Vision Sciences, Glasgow Caledonian University, Scotland. Antioxidant supplements and placebos were kindly donated by Vitabiotics.
Descriptors: marginal dry eye; tear stability; tear volume; conjunctiva; antioxidants; vitamins
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Introduction

The health and optical clarity of the cornea of the human eye are of great importance as detriment may lead to degradation or loss of vision. Although exposed to the atmosphere between blinks, the anterior ocular structures of the cornea and the conjunctiva are protected by a well organised film of tears. The tear film is essentially a trilaminar structure comprising a deep mucus layer produced primarily by the goblet cells of the conjunctiva which increases ocular surface wettability; a substantial aqueous layer produced by the main and accessory lacrimal glands and a superficial layer of oil from the Meibomian glands in the eyelids which inhibits. The dynamic tear film structure is refreshed with the sweeping action of the eyelids on each blink.

Under normal conditions, the tear film is of sufficient quantity and quality to establish a refractive surface of high quality for the cornea and to ensure the well-being of the corneal and conjunctival epithelium (Holly, 1980). Abnormalities in the production, quality or replenishment of the tear film will result in various pathological states regarded as dry eye conditions. Such conditions can result in ocular surface damage, and may lead to eventual corneal damage which could impair corneal transparency and visual performance. The term ‘dry eye syndrome’ describes a variety of conditions, of mixed aetiology but sharing common subjective symptoms and objective clinical signs, leading to a physical and functional break-down of the tear film (Lemp, 1995).

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Contributors: KJB conducted the study and collected all data and analysed them. He did the major work in writing the manuscript. SP randomised the subjects' treatment orders and supervised the study. KEA advised regarding the micronutrients of the antioxidants and co-supervised the study.
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Such tear film disorders range in severity, from the borderline dry eye, which may only be apparent under conditions such as environmental challenge (McMonnies, 1986), to the severe (pathological) dry eye (keratoconjunctivitis sicca, KCS), as often found in Sjögren's syndrome. Dry eye problems may be the result of low tear volume (aqueous deficiency), or inadequate quality (lipid deficiency or mucus deficiency). Any factor or pathology which damages the surface of the cornea or conjunctiva, or disrupts the structure and shape of the eyelids is also likely to promote dry eye.

The involvement of nutritional components in the aetiology of some dry eye states has been reported (Sommer & Muhialal, 1982; Sullivan *et al*, 1973). Vitamin A is important for the integrity and function of the corneal and conjunctival epithelial cells and its deficiency may promote abnormal conjunctival changes (van Agtmaal *et al*, 1988; Udomkesmalle *et al*, 1992). While vitamin A deficiency is a rare condition in the Western world, it is found under some conditions, such as chronic liver disease, cystic fibrosis, regional enteritis and other causes of diet restriction or poor absorption (Petersen *et al*, 1968; Russell *et al*, 1973; Sullivan *et al*, 1973; Smith *et al*, 1975).

Detrimental conjunctival changes are known to be an early consequence of vitamin A deficiency (Natadisastra *et al*, 1987) and conjunctival effects of nutritional deficiency have been shown to be reversible with systemic vitamin A therapy (Sommer, 1983).

Patel *et al* (1993) demonstrated a significant increase in tear film quality (stability) following supplementary multivitamin intake by a normal Western population. In terms of improved tear stability they reported that the synergistic effects of a multivitamin treatment were more predictable than the effects of a single nutritional component (vitamin C) alone. Similarly, combined supplementation of zinc and vitamin A promotes a better reversal of conjunctival changes in non-xerophthalmic patients with suboptimal nutriture, than zinc or vitamin A alone (Udomkesmalle *et al*, 1992). These findings imply that nutritional influences on tear film composition and physiology are complex. The findings of Patel *et al* (1993) are of particular importance because low tear film stability is a common sign and consequence of many dry eye conditions. If the effect noted in normals extends also to dry eye sufferers, then nutritional supplements could be used as a convenient treatment.

Dry eye has previously been treated using artificial tear substitutes to address tear volume or quality issues (Foulkes, 1998). Such prophylactic treatments may offer transient relief of symptoms, but must be repeated frequently as required (Swanson, 1998). Others have attempted to address tear insufficiency problems by occluding the puncta, the channels which normally drain the tears from the surface of the eye into the naso-lacrimal system for clearance (Murube & Murube, 1996). While this is performed to increase the volume of tears present at the ocular surface, there is evidence that the lacrimal system can reduce tear production in response to punctal occlusion, presumably through a feedback mechanism which normally prevents epiphora in normals (Tomlinson *et al*, 1998). While punctal occlusion may benefit aqueous deficient patients, it may not be a suitable treatment for patients who are not aqueous deficient, but suffer from another form of dry eye.

It has been proposed that prospective clinical trials should be conducted to assess the efficacy of vitamin supplements for treating non-Sjögren's syndrome dry eye (Foulkes, 1998). However, most of the published investigations have used topical vitamin-containing eye drops (Holly, 1993).

The purpose of this study was to assess whether an orally administered antioxidant dietary supplement could improve the objective clinical signs and alleviate the subjective symptoms of marginal dry eye in a Western population.

Experimental design

A prospective, cross-over, placebo-controlled, randomised, predominantly double-masked clinical trial design was adopted, whereby each subject was evaluated at baseline (prior to any intervention) and again at monthly intervals, following each of three treatment periods. The treatment periods were: (i) no treatment given for 30 days; (ii) placebo treatment given for 30 days, and (iii) oral antioxidant supplements given for 30 days. Treatments were assigned in random order, by the method of Latin squares (Fleiss, 1986).

This trial was double-masked for the periods of placebo treatment and antioxidant supplements, but only single mask for the period with no treatment as there was no way of masking the subjects to the lack of treatment in this period.

The treatment was an antioxidant complex product called VisionACE® (Vitabiotics, London, UK). This is a commer-

Table 1 Visionace® (Vitabiotics, London, UK)

Constituent	*Average per two capsules*
β-Carotene	6 mg
Vitamin E (natural source)	120 mg
Vitamin C	300 mg
Vitamin B6	30 mg
Vitamin D (200 IU)	5 µg
Thiamin (vitamin B1)	15 mg
Riboflavin (vitamin B2)	10 mg
Vitamin B12	9 µg
Folacin (as folic acid)	500 µg
Vitamin K	200 µg
Pantothenic acid	20 mg
Magnesium	100 mg
Zinc	15 mg
Iron	6 mg
Iodine	200 µg
Copper	2 mg
Manganese	4 mg
Selenium	200 µg
Chromium	100 µg
Cystine	40 mg
Methionine	40 mg
Bioflavinoids	30 mg

Table 2 From McMonnies' dry eye questionnaire

Questions regarding symptoms of dry eye that are constantly experienced:

2. Do your ever experience any of the following symptoms? (Please *underline* those that apply to you)
    1. Soreness
    2. Scratchiness
    3. Dryness
    4. Grittiness
    5. Burning
3. How often do you have these symptoms? (*Underline*)
    - Never
    - Sometimes
    - Often
    - Constantly

Questions regarding symptoms of dry eye that are experienced in response to provocative stimuli:

4. Are you unusually sensitive to cigarette smoke, smog, air conditioning, or central heating?
    - Yes
    - No
    - Sometimes
5. Do your eyes easily become very red and irritated when swimming?
    - Not applicable
    - Yes
    - No
    - Sometimes
6. Are your eyes dry and irritated the day after drinking alcohol?
    - Not applicable
    - Yes
    - No
    - Sometimes

cially available product, in the UK. The active ingredients of VisionACE® are given in Table 1. The placebos for the study were of identical colour, texture, shape and size, however they contained only starch powder. The placebo capsules were also manufactured and supplied by Vitabiotics.

Prior to administration to the subjects, the capsules were hygienically loaded into standard unmarked tablet bottles, labelled with a random five digit identifier code. These codes were not broken until the completion of the study.

Experimental procedures

To assess the signs and symptoms of marginal dry eye, several tests were employed. Tests were included to assess tear volume and quality, health of the underlying dependent ocular tissues, and severity of dry eye symptoms experienced.

The tests were performed in a standard order to prevent any one procedure influencing the results of subsequent tests. The order was Tear Thinning Time (TTT); Glasgow Caledonian University Thread (GCUT) test; Dry Eye Questionnaire; Conjunctival Impression Cytology.

Subject recruitment

The required individuals were sufferers of marginal dry eye, complaining of ocular discomfort or having low tear stability. Only subjects not receiving any conventional treatment for the condition, such as artificial tears, were recruited. The subjects were recruited proactively, through the Glasgow Caledonian University eye clinic, with a questionnaire screening to identify patients suffering dry eye symptoms. Additional marginal dry eye sufferers referred by optometric practitioners and ophthalmologists were also accepted, provided they fulfilled the standard recruitment criteria and were not receiving any dry eye treatment.

Subject selection criteria

The selection criteria were set to allow broad intake of marginal sufferers. Individuals were enrolled as subjects if: (i) they had a non-invasive TTT of under 10 s; and/or (ii) their McMonnies dry eye questionnaire responses at initial contact were considered indicative of significant primary or secondary dry eye symptomology (Blades & Patel 1996b).

Tear quality (stability)—tear thinning time (TTT)

Tear stability tests investigate the ability of the tear film to adequately cover the otherwise exposed anterior surface of the eye, for a sufficient duration of time to prevent drying and subsequent damage to the underlying tissues. The tear film is respread and reformed with each blink (van Haeringen, 1981). Spontaneous blinks occur every 4–5 s in normal eyes (Patel *et al*, 1991; Zaman *et al*, 1998). Tear film stability is taken to be insufficient if break-up occurs in under 10 s (Farrell *et al*, 1992). Tear film stability assessment techniques can be considered as invasive or non-invasive, with non-invasive tests such as the HIRCAL grid considered to give more valid results (Craig & Blades, 1997). The HIRCAL grid measures the time taken from a blink until the tear film begins to show irregular thinning, which happens just before the tear film ruptures, exposing the underlying epithelium. The HIRCAL grid is a modified Bausch and Lomb keratometer, with the original mires replaced by a plate consisting of a white grid etched on a black background. The illuminated pattern of horizontal and vertical white lines was projected onto the tear film surface. The image of this grid was observed following a blink, for the first sign of a discontinuity or distortion of the white lines. At this point, the time was recorded as the TTT. This was repeated five times and the average TTT was calculated (Hirji *et al*, 1989).

Tear volume—the Glasgow Caledonian University threads (GCUT) phenol red thread wetting

The GCUT phenol red thread test uses a fine, pH-indicator-impregnated thread to measure the amount of tears absorbed from the lower lacrimal lake in unit time. The test was performed by dipping the end of a fine thread over the lower eyelid into the tear meniscus, and leaving it place for 2 min. After this the extent of thread wetting was measured as the alkaline tears cause the wetted portion of the thread to change colour from light yellow to red (Blades & Patel, 1996a). Previous work with this particular thread test has shown that the extent of thread wetting is lower for a group of aqueous deficient patients than for non-aqueous deficient normals (Patel *et al*, 1998).

Dry eye symptom perception

Subjective symptom assessment was performed, using McMonnies' dry eye questionnaire (McMonnies & Ho, 1987a,b). This contains several questions pertaining to the perception of the ocular symptoms of dry eye. In addition to providing a total questionnaire score, it was possible to compute scores to describe the perception of constantly experienced dry eye symptoms and symptoms experienced in response to provocative stimuli such as environmental factors (Blades & Patel, 1996b).

Ocular surface status—Squamous metaplasia assessment and goblet cell density

Conjunctival impression cytology (CIC) is a method of investigating surface damage in dry eye, at a cellular level (Lemp, 1995). The test involved pressing a small Millipore© filter against the conjunctiva to collect a sample of conjunctival epithelial cells which were fixed, stained and examined by light microscopy. By this technique, two parameters were investigated: (i) the goblet cells that produce much of the basement mucus of the tear film were selectively visualised by the Periodic acid-Schiff reaction (PAS). The number of goblet cells per unit field of view (0.6 mm^2) was calculated (Nelson & Wright, 1984; Rivas *et al*, 1993); and (ii) appearance of and degree of squamous metaplasia. Haematoxylin was used to visualise the other epithelial cells collected. These were compared to a photographic scale of increasing ocular surface damage (Tseng, 1985).

Statistical analyses

Data which were of Gaussian distribution (Shapiro–Wilk test, $P > 0.05$) were analysed using parametric analysis of variance (one-way ANOVA); and Student's *t*-test and were expressed as a bar graph. Non-Gaussian data were analysed using distribution-free statistical tests and expressed as box plots. In these, the medians are represented by horizontal bars: the 25–75th percentiles are enclosed by boxes; the 10–90th percentiles are enclosed by vertical bars and the remaining 20% (extreme) data points are shown as hollow circles. Factorial analysis (Student's *t*-test and the Mann–Whitney test) was employed to compare the results.

Results

Forty marginal dry eye sufferers with poor tear stability and/or perceived dry eye symptomology were recruited for this trial. This subject group was composed of 30 females and 10 males, with a mean age of 53.4 ± 15.3. Initial diagnostic parameters were: (i) mean GCUT wetting was 16.4 mm with a standard deviation of 6.8; (ii) median TTT was 7 s with a range of 1.2–20; (iii) median goblet cell population density was 7.7 per 0.6 mm^2 with a range of 0–66.7; (iv) median Tseng's squamous metaplasia scale score was 1.5 with a range of 0–5; (v) median total McMonnies' dry eye questionnaire score was 13 with a range of 4–22; (vi) median provoked dry eye symptom score (derived from McMonnies' dry eye questionnaire responses) was 2.5 with a range of 0–5; and (vii) median provoked dry eye symptom score

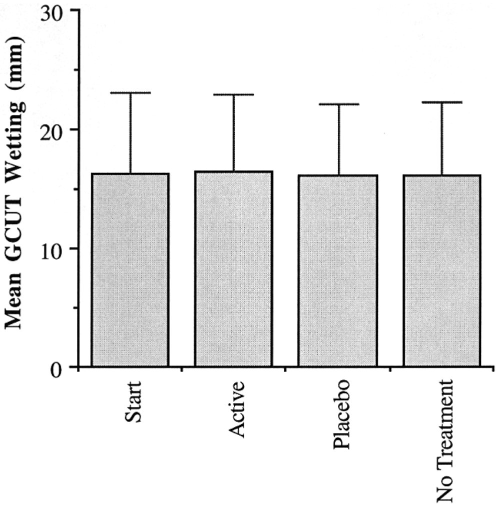


Figure 1 The mean (±s.d.) G-CUT wetting prior to any treatment, and following each of the treatment periods.

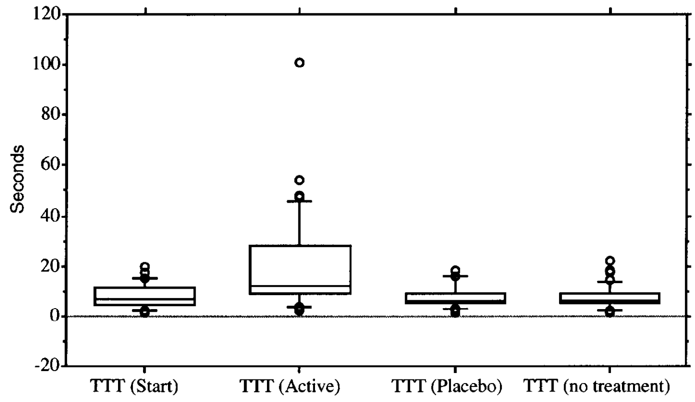


Figure 2 'Box and Whiskers' plot representing the spread of tear thinning times (TTT) recorded following each treatment.

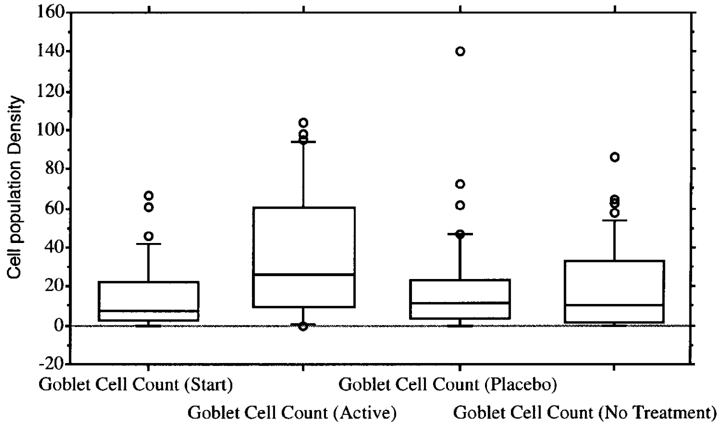


Figure 3 The distribution of goblet cell density data collected prior to any treatment and following each of the treatment periods.

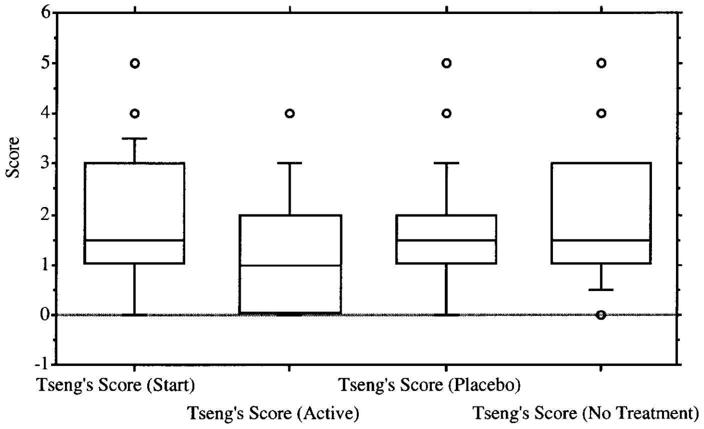


Figure 4 The distribution of Tseng's metaplasia scale score data collected prior to any treatmdent and following each of the treatment periods.

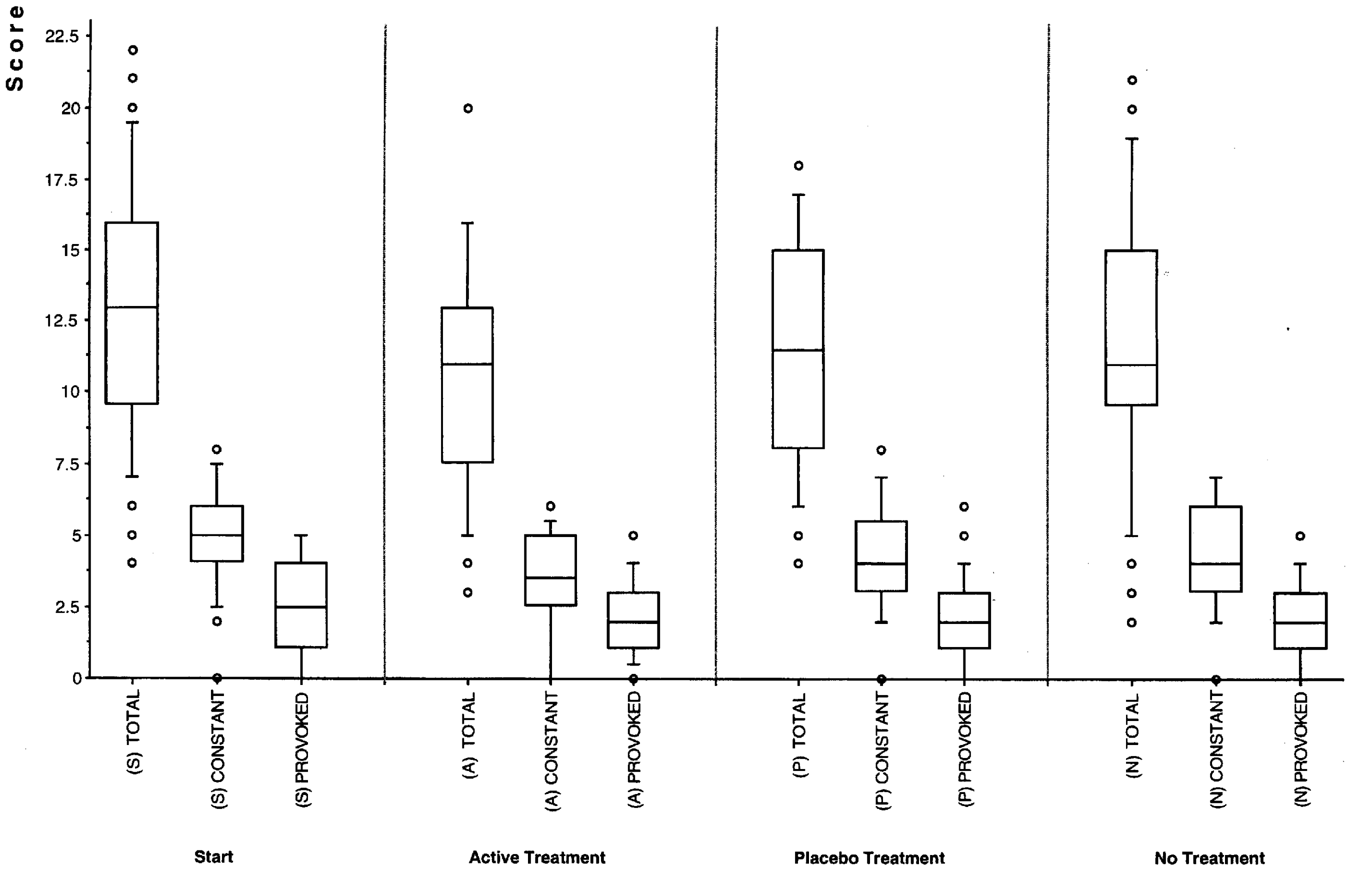


Figure 5 Symptom responses following each of the treatments.

(derived from McMonnies' dry eye questionnaire responses) was 5 with a range of 0–8.

Figures 1–5 present the GCUT thread wetting, TTT, conjunctival status and subjective symptom data from baseline and following each of the treatment periods.

The effect of no treatment

After no treatment was given to the subjects for 1 month, there was no significant difference in any of the objective or subjective parameters assessed ($P > 0.05$).

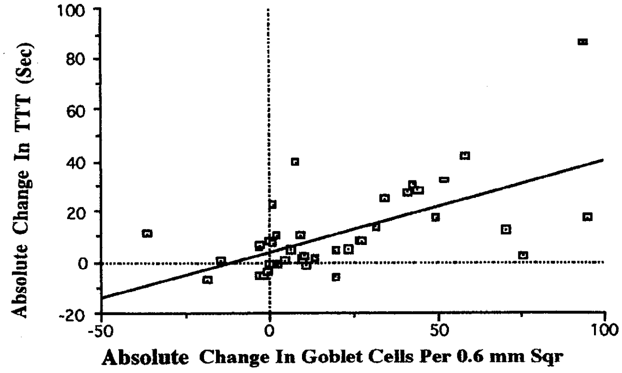
The effect of placebo treatment

After the placebo treatment was given to the subjects for 1 month, there was no significant difference in any of the objective parameters assessed ($P > 0.05$). However, the subjects' subjective reports regarding constantly perceived dry eye symptoms were significantly improved from baseline reports, following the placebo treatment (Mann–Whitney, $P = 0.0411$), although the provoked symptom scores were no different after the placebo treatment period ($P > 0.05$).

The effect of antioxidant treatment

The extent of GCUT thread wetting was not significantly different from baseline, following 1 month of active antioxidant treatment ($P > 0.05$). However, significant improvements were found in TTT (Wilcoxon's signed rank test, $P = 0.0001$); goblet cell density (Wilcoxon's signed rank test, $P = 0.0001$) and squamous metaplasia (Wilcoxon's signed rank test, $P = 0.0044$), following this treatment period.

TTT before and after antioxidant treatment was also plotted, and a 'line of no change' added, to indicate the clinical relevance of the change in tear stability mediated by the antioxidant treatment period (Figure 6). The majority of subjects (80%) demonstrated some increase in tear stability following the antioxidant treatment period. Of the subjects who had a TTT of under 10 s at baseline, 92% had improved tear stability and 60% had a tear stability of over 10 s following the antioxidant treatment period. Of the subjects



A Significant Spearman's Rank Correlation Was Found Between The Absolute Change In Tear Thinning Time And The Absolute Change In Number Of Goblet Cells Found In A 0.6 mm^2 Area Of Conjunctiva, Following The Active Treatment Period (cc=0.568)

Figure 6 Absolute changes in TTT and goblet cells in the subjects.

who had a TTT of under 5 s at baseline, 84.6% had improved tear stability and 30.8% had a tear stability of over 10 s following the antioxidant treatment period. Of the subjects who had a TTT of between 5 and 10 s at baseline, 91.7% had improved tear stability, and all 91.7% had a tear stability of over 10 s following the antioxidant treatment period.

'Carry over effect'

To assess whether a 'carry over effect' (sustained activity of the active treatment, without the active treatment period) had influenced the results collected following the placebo treatment period, treatment orders were divided into those where the active treatment was administered immediately prior to the placebo treatment, and those where it was either administered after the placebo period or another treatment separating the active and placebo treatment periods. The data analysed using Mann–Whitney test were: (i) TTT; (ii) conjunctival hyperaemia; (iii) subjective symptom perception; (iv) conjunctival epithelial squamous metaplasia; and (v) conjunctival goblet cell population density. Data from GCUT wetting were analysed using Student's *t*-test. The *P*-values of these analyses for all groups were over 0.05, indicating that no carry-over effects affected the parameters assessed following the placebo treatment period. The activity of the treatment given therefore did not mediate any changes which persisted for 1 month following cessation of active treatment.

A significant correlation was found between the absolute changes from baseline in TTT and goblet cell densities following the antioxidant treatment period (Figure 7; Spearman's rank correlation coefficient $= 0.568$; $P < 0.002$). The subjects' subjective reports regarding constantly perceived dry eye symptoms were again significantly improved from baseline reports following the active treatment (Mann–Whitney, $P = 0.001$), but provoked symptom scores were no different following this treatment period ($P > 0.05$).

Discussion

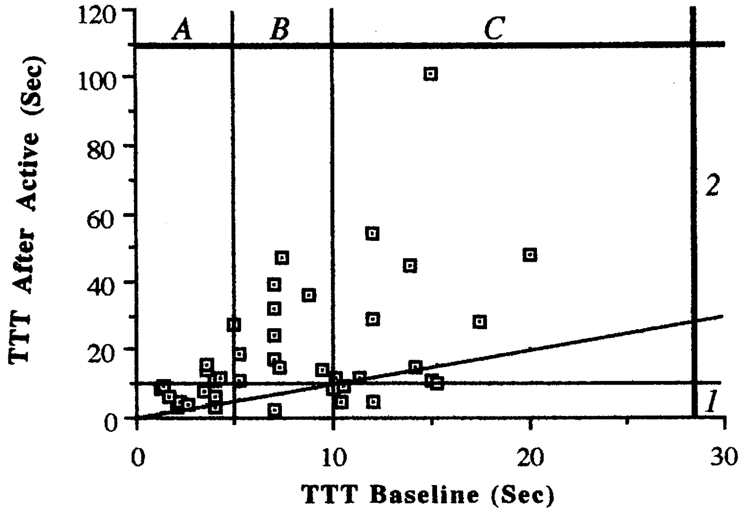
The results demonstrate that the orally administered antioxidant dietary supplements given improve the tear film stability and the health of the conjunctival surface in marginal dry eye sufferers. No net improvements in tear volume were found. True improvement in subjectively reported symptoms was not found, because the subjective symptoms were improved when the placebo treatment was given.

The tear film/anterior eye is in a very dynamic relationship and Patel *et al* (1993) showed a significant increase in tear film stability with nutritional supplements for 10 days.

The most consistent and clinically important improvement was the increase in tear stability in subjects who had a TTT of between 5 and 10 s at baseline. These subjects would initially have been indicated as having poor tear stability (Farrell *et al*, 1992), but 91.7% of them had a tear stability of over 10 s, which would be classed as normal, following the antioxidant treatment period.

We detected a significant correlation in the increased tear stability and the increased conjunctival health. However, we cannot determine if tear stability improved as a direct consequence of an increase in conjunctival health and goblet cell count or vice versa.

Several mechanisms, could explain the clinical improvements mediated by the antioxidants, both individually or in concert. Both the nutritional and the antioxidant properties of the dietary supplements given could explain the results. For example, vitamin A is known to be involved in epithelial differentiation and as such is an important micro-



A Subjects With A Tear Thinning Time Of Under 5 Seconds Prior To Any Treatment.
B**:** Subjects With A Tear Thinning Time Of Between 5 And 10 Seconds Prior To Any Treatment.
C**:** Subjects With A Tear Thinning Time Of Over 10 Seconds Prior To Any Treatment.
1**:** Subjects With A Tear Thinning Time Of Under 10 Seconds Following Oral Antioxidant Therapy.
2**:** Subjects With A Tear Thinning Time Of Over 10 Seconds Following Oral Antioxidant Therapy.

Data Points Which Lie In The *B2* Area Of This Graph Indicate Individuals With An Initial Tear Thinning Time Of 5-10 Seconds, Who Had A Tear Thinning Time Of Over 10 Seconds Following Oral Antioxidant Therapy.

Figure 7 TTT of subjects prior to and after treatments.

nutrient, essential for the development of the mucus producing goblet cells of the conjunctiva. The antioxidant properties of vitamin A, that is, its ability to combat free radicals at physiological pO$_2$ may also assist in the maintenance of the conjunctival tissues, and thus afford greater stability to the tear film (Burton *et al*, 1985). Similarly, the lacrimal glands and the Meibomian glands are also composed of epithelium. Vitamin A may be important in these tissues also. Thus, vitamin A may help to maintain the tissues responsible for tear film production, so may increase the quality of the tears produced by the marginal dry eye.

Free radicals, being inherently unstable, effectively attack and damage critical cellular components, notably plasma membranes, in an attempt to complement their unpaired electrons. This can cause cell damage and death in a number of ways, one of the most commonly cited actions being lipid peroxidation leading to cell membrane damage, uncontrolled ionic flux and cell lysis (Comporti, 1993). Hence, free radicals can damage epithelial tissues such as the conjunctiva, the lacrimal glands and all tear-secreting tissues.

Normal tear components including hyaluronate and proteins, which may promote tear stability (Schoenwald *et al*, 1998), are degraded by airborne pollutants including ozone (Rohen & Lutjen-Drecoll, 1992; Schmut *et al*, 1994).

Thus, airborne pollutants may compromise the tear film. Extreme or extended exposure to O$_3$ may deplete the tear proteins, thus exhausting this protective system, so causing chronic dysfunction of the tear film. This may account for a subtype of dry eye problems, which should be termed as an 'air-pollution-induced dry eye syndrome' (Schmut *et al*, 1994).

The tear film of the questionable dry eye has reduced levels of some proteins (Seal, 1985), so may possess inadequate defences against oxidative stress, mediated by such factors as environmental pollutants. Intake of nutritional antioxidant supplements may fortify the remaining tear film and ocular surface antioxidant defences.

It is proposed that the improvement in marginal dry eye ocular surface health found in this investigation was mediated by the increased tear stability afforded by the supplementary antioxidant intake, which spared tear film components such as proteins from environmentally mediated oxidative stress.

Dry eye is a condition defined by and often investigated clinically in response to subjective symptoms. It is unfortunate that a placebo effect was found in this investigation. This highlights the need for objective clinical tests when assessing outcomes of dry eye therapy.

In conclusion, the antioxidant treatment given improved tear stability and conjunctival health, but did not promote a net increase in tear volume in marginal dry eye sufferers. These clinical improvements may have been promoted by sparing tear film components from oxidative stress. The most consistent, clinically relevant improvements were noted in marginal dry eye sufferers initially presenting with TTT of between 5 and 10 s. Although dry eye symptom perception is an important indicator of dry eye, it is not a reliable indicator of dry eye therapy efficacy. For a more reliable clinical monitoring of the dry eye condition and its progress tear stability should be assessed.

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