



AN ANALYSIS OF THE PROTEIN AND LIPID CONCENTRATIONS IN THE TEAR LAYER OF CONTACT AND NON-CONTACT LENS WEARERS.

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ABSTRACT

Purpose: The main reason for embarking upon this specific aspect of the study is to obtain local population statistics in Trinidad, W.I. Most of the research pertaining to the tear layer has been done internationally with statistical analysis stemming from the Western world. The clinical significance of this study is to provide early means of detection of primary and secondary diseases such as hyperlipoproteinemia, dyslipidemia, and triglyceridemia in human population. The significance is to determine the relationship between a contact lens wearer and individual components (total proteins and total lipids) of their tears. This is to examine the tear-lens relationship between the deposition of components of the tear film on soft (hydrogel and silicone hydrogel) contact lenses in relation to lens intolerance, patient discomfort and dry eye syndrome.

Method: A total of 93 subjects were interviewed and tear samples were obtained from 81 participants. The capillary tube technique was used to extract tear fluid samples and were stored at -80°C. Total Protein and Triglyceride Liquicolour kits were used to analyse tears. Using a mass spectrometer, absorbance values were used to calculate the total protein and total triglyceride concentrations for non-contact lens wearers and contact lens wearers.

Results: It was formulated that the total concentrations of proteins and lipids in the tear film of non-contact lens patients were 7.56±1.31 and 3.76±3.26 mg/dl. The protein and lipid concentrations of contact lens patients were also determined; these results were 5.88±0.91 and 2.54±0.72 mg/dL.

Conclusion: Based on independent statistical analysis, there was no significant difference between the protein and lipid concentrations of non-contact lens patients. There was also found to be no significant difference between contact lens and non-contact lens wearers' protein and lipid concentrations.

HYPOTHESIS

Alternate Hypothesis (H₁): The lipid concentration in the tear film of non-contact lens patients is higher than in contact lens patients, however the protein concentration is higher in contact lens patients than in non-contact lens patients.
Null Hypothesis (H₀): The protein and lipid concentrations in the tear film of both contact and non-contact lens patients are the same throughout.

AIMS/OBJECTIVES

- To determine the tear fluid components (total proteins and total lipid concentrations) in Non-contact lens wearers and Contact lens wearers individually
- To investigate the difference in both concentrations between Non-contact lens wearers and Contact lens wearers

INTRODUCTION

The anatomy of the lacrimal system begins with tears. Tears are clear bodily fluids, which are formed, in lacrimal ducts within the eye. Tear secretion occurs 1.2 per minute and total tear volume amounts to 7. The basis of the tears is the tear film. The tear film is also divided into three principal layers; the lipid layer, the aqueous layer and the mucous layer. The tears function mainly to lubricate and moisten the ocular surface. They also facilitate non-frictional movements between the eyelid and the globe allowing for smooth surface movements when blinking. Tears also cleanse the eye of waste, protect from infection and have antibacterial and anti-inflammatory properties. This keeps an optically smooth surface of the eye. The corneal surface is hydrophobic and tears lubricate and therefore allow an ambient flow of oxygen through it¹. There are two types of tears that exist, Basal tears and Reflex tears. Basal tears keep the cornea continuously moist and help clear out dust and foreign bodies. These tears are constantly manifested and are secreted by the accessory lacrimal glands of Krause and Wolfring. Reflex tears are tears that result when the eye is irritated or contains any foreign substance. Some of the main stimuli that activate reflex tears are dust, light and spicy foods. These are only secreted when these situations occur and are secreted by the Main Lacrimal Gland. For the purposes of this study, no specific tear type was extracted, although based on the nature of the method of tear extraction, we expected mostly reflex tears. The significance of determining proteins and lipids in tears is extremely vital when deducing different ocular conditions in the average healthy individual. Interaction between the contact lens and the tears are affected by lens composition and lens material properties apart from the tear film of the individual patient and their scheduled wearing time.³ A good tear film is necessary for the tolerance of a contact lens. When the tear film over the contact lens, aqueous from the tear film evaporates between blinks allowing solid deposits from the tears to coat the lens surface. Lipid and protein deposits alter the uniformity of the lens surface thereby resulting in an irritated eye and lens intolerance. Deposition and denaturation of protein components stimulate the immune response. Hydrogel contact lenses are susceptible to protein build up. Silicone Hydrogel material contact lenses have an increased lipid interaction and deposition.²

METHODOLOGY

The patient was chosen from the UWI Optometry Clinic in St. Augustine. The patient was identified based on the inclusion and exclusion criteria of this study. Therein the subject chosen was between the ages of 18-40 years old. This specific age range was chosen because 18-34 is the range of most healthy individuals. Persons who wear contact lenses were used in this study since different types of contact lenses worn over a period of time may affect the corneal integrity and have an effect on the Dk/t values, which can impact the tear production. Based on a statistical analysis calculation, a predicted sample size of 200 for each sample type. However, due to external factors only 88 were obtained. After the patient was identified they gave written consent to partake in the study via the form they completed, signed and dated. This allowed me to be certain that they were competent and were fully aware of the proceedings that took place during the extraction of the tear samples. A questionnaire was then filled out by the subject. The sample was then collected from each subject using a microcapillary tube. Hands were washed, and gloves worn. Using the forefinger, the lower eye-lid was gently pulled vertically downward just enough to obtain a proper visual of the conjunctival sac. Their tears were collected into the Eppendorf and labelled with a UIN code. After which they were insulated and placed into a -80°C freezer until they were ready to be analysed using a Total Triglyceride Liquicolour and Total Protein Liquicolour Kits. Three major reagents were used during this procedure: the biuret reagent which contains 15% copper sulphate and 3% sodium hydroxide, total protein blank reagent which contains 3% sodium hydroxide, protein and triglyceride standard solution. Patient tear samples were removed from -80°C thawed to room temperature. Reagents, Standard and Controls were respectively placed and mixed in each test tube along with tear sample. 10µl were used for lipid analysis and 20µl of tear sample was used for proteins. These were further mixed and placed into cuvettes where absorbance values for proteins and lipids were obtained using Mass Spectrometry. Formulas were then used to calculate respective concentrations. Samples were analysed in batches of 20.

RESULTS

Sample Type	Total Protein Concentration (g/dL)			Total Lipid Concentration (mg/dL)		
	Mean ± Standard Error	Standard Deviation	P-value	Mean ± Standard Error	Standard Deviation	P-value
Contact Lens Wearer	5.8883 ± 0.91203	4.37393	0.937	2.5403 ± 0.72000	3.45298	0.271
Non-Contact Lens Wearer	7.5657 ± 0.53464	4.47311		3.7621 ± 1.33829	11.19698	

Table 1 Showing the results obtained from a T-Test for the comparison of Contact Lens Wearers and Non-Contact Lens Wearers and their Total Protein Concentration (g/dL) and Total Lipid Concentration (mg/dL)

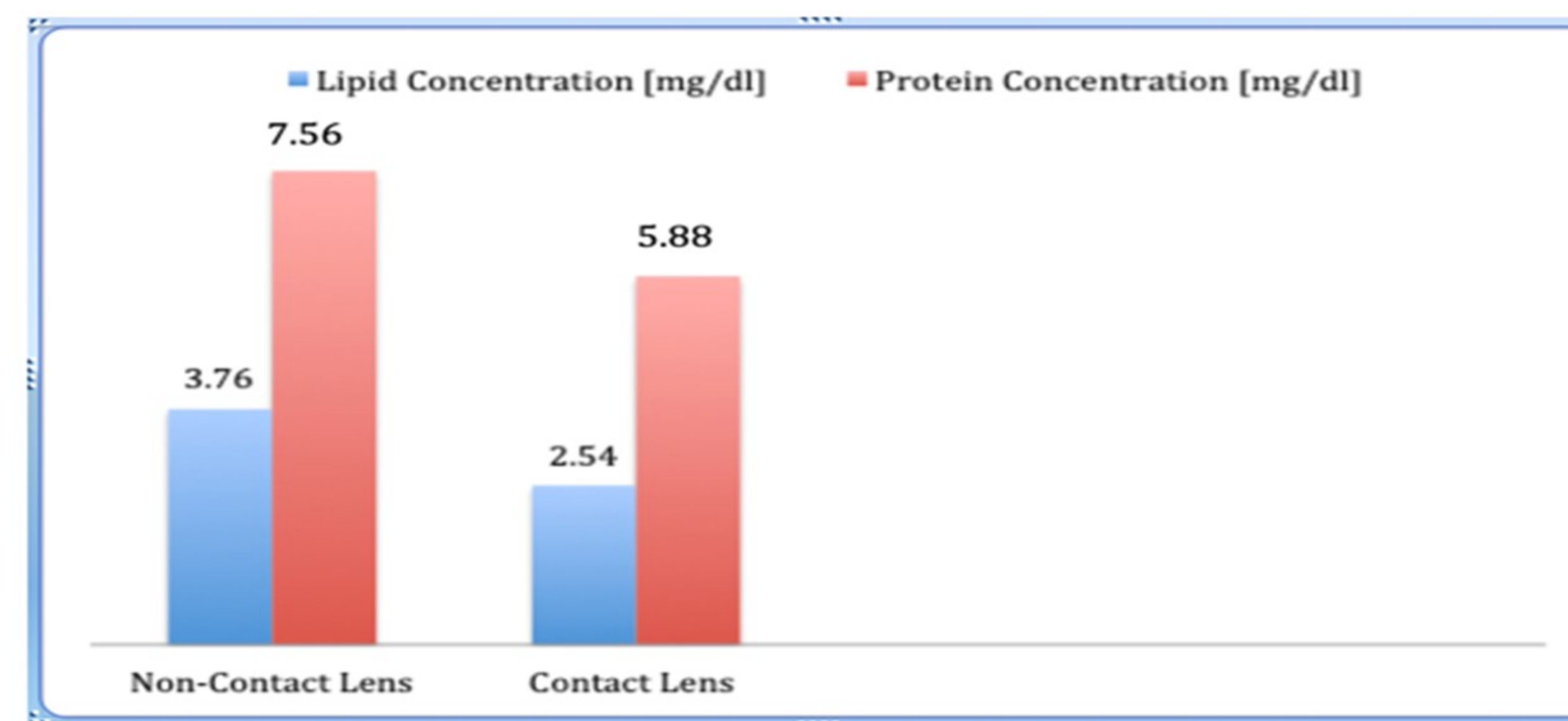


Figure 1. A bar graph representing the lipid and Protein concentrations of both sample types.

DISCUSSION

The lipid layer of the tear film is the outermost and thinnest layer which functions mostly in retaining moisture. Important proteins are scattered throughout this layer, which aids in its functions. Therefore, biology would suggest that if the lipid layer were compromised, it would not be fully able to fulfil its function. Based on the data collected after the analysis of the tears, the mean concentrations for proteins and lipids were found to be; 7.56 mg/dl and 3.76 mg/dl. The P-values of these two variables were found to be 0.27 and 0.93, given that both were more than 0.05 within a 95% Confidence Interval. The difference between the protein and lipid concentrations in a non-contact lens patient was found to be clinically insignificant. The results of this study with Non-contact lens patients were also compared to the protein and lipid concentrations of Contact lens patients. An Independent T-test used to statistically analyse the values. It was concluded that since the P-values of both variables were 0.60 and 0.12 which is less than 0.05 within a 95% Confidence Interval, the differences between the protein and lipid concentrations of contact and non-contact lens patients is clinically insignificant. Therefore, we can deduce that contact lens materials have no effect on the stability of the layers of the tear film regarding lipids and proteins when compared to the tear film of persons who do not wear contact lenses. Based on the Alternate hypothesis, higher lipid concentrations were expected in non-contact lens patients since this tear film layer would be uncompromised by a hydrogel or silicone hydrogel contact lens. The lipid layer would not be disrupted and therefore normal concentrations of lipids would seemingly be present. Higher protein concentrations were expected in contact lens patients since our eyes were not anatomically made for a contact lens. The body would, therefore, perceive it as a foreign body and activate its second chemical line of defence which mostly involves proteases, lactoferrins and immunoglobulins which are predominantly proteins. However, the results of this study disprove this hypothesis since there was no statistical difference between these concentrations. This insinuates, that contact lenses are perfectly fine for the eye and causes no disruption to the tear film. If problems arise while wearing them, the inconsistency in the tear layer is not the cause.

CONCLUSION

The mean protein and lipid concentrations in non-contact lens patients were found to be 7.56 ± 1.31 mg/dl and 3.76±3.26 mg/dl. No difference was found between these concentrations. The difference in concentrations of lipids and proteins in non-contact lens patients compared to contact lens patients were also found to be insignificant. The Alternate hypothesis was therefore disproved.

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