Associations of Biomechanical Properties of the Cornea With Environmental and Metabolic Factors in an Elderly Population: The ALIENOR Study

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PURPOSE. The purpose of this study was to assess the associations of biomechanical properties of the cornea with metabolic and environmental factors in an elderly population.

METHODS. The ALIENOR (Antioxydants, Lipides Essentiels, Nutrition, and Maladies OculaiRes) study is a population-based study. In 2009–2010, 624 subjects, aged 74 years or more, underwent an eye examination, including intraocular pressure (IOP), central corneal thickness (CCT), and biomechanical properties of the cornea measurements using the Ocular Response Analyzer. Socio-demographic, lifestyle, and medical history data were collected using standardized questionnaires. Mean lifetime ambient ultraviolet (UV) exposure was estimated using residential history and statistics of UV radiation at each location using the Eurosun UV database.

RESULTS. Mean age was 82.2 ± 4.3 years. Mean corneal hysteresis (CH), corneal resistance factor (CRF), and CCT were 9.4 ± 1.9, 9.8 ± 1.9 mm Hg, and 551.6 ± 36.8 μm, respectively. In the multivariate analysis, CH and CRF values were significantly lower in subjects older than 80 years (-0.56; 95% confidence interval [CI]: $-0.89; -0.24$); $P < 0.001$ and -0.48; 95% CI: $-0.75; -0.20$; $P < 0.001$, respectively), in subjects having higher ambient UV exposure (-0.50; 95% CI: $-0.88; -0.12$; $P < 0.01$; and -0.46; 95% CI: $-0.78; -0.13$); $P < 0.05$, respectively), and in subjects with high plasma LDL cholesterol (CH: -0.46; 95% CI: $-0.86; -0.03$; $P < 0.05$; and CRF: -0.37; 95% CI: $-0.72; -0.008$; $P < 0.05$). Central corneal thickness was significantly higher in former smokers than in never smokers ($+11.01$; 95% CI: $0.48; 21.55$; $P < 0.05$) and was not significantly associated with age, ambient UV exposure, diabetes, or LDL cholesterol.

CONCLUSIONS. Biomechanical properties of the cornea are modified by metabolic and lifetime environmental factors, especially UV exposure. The manner these factors may influence onset and progression of ocular diseases or IOP measurements need further investigation.

Keywords: biomechanics, corneal elasticity, corneal hysteresis, corneal biomechanical properties, aging, UV exposure

Cornea ultrastructure is the result of the interaction between a precise diameter and orientation of fibrillar collagen bundles and the other components of the extracellular matrix mainly represented by proteoglycans and glycosaminoglycans responsible for the regulation of collagen spacing.[1] This highly differentiated ultrastructure of the corneal tissue leads to its viscoelastic deformation properties and its biomechanical behavior.[2] In clinical practice, biomechanical properties of the cornea can be estimated using the Ocular Response Analyzer (ORA; Reichert, Inc., Depew, NY, USA), which evaluates deformation response of the cornea under loading and unloading pressure using a calibrated air-puff.[3]

Although central corneal thickness (CCT) is an important influencing factor of intraocular pressure (IOP) measurements, corneal biomechanical properties also significantly affect its

accuracy.[4–7] Additionally, it has been demonstrated that biome-chanical properties of the cornea are altered in some corneal diseases such as keratoconus or postoperative corneal ectasia.[8,9]

Whereas quite a few studies have highlighted the potential role of biomechanics on the pathophysiologic process of some ocular diseases and how they can influence IOP measurements, little is known about their associations with most frequent metabolic or environmental factors observed during lifetime. Such modifications of the ultrastructure of the cornea and changes of its biomechanical behavior with age or metabolic factors might at least partially mediate the associations of these factors with glaucoma or corneal diseases.[10–18]

Furthermore, to our knowledge, the influence of ultraviolet (UV) exposure—one of the most frequent environmental

hazards during lifetime—on the thickness and biomechanics of the cornea has never been explored.

We therefore investigated the associations of most frequent metabolic and environmental factors on biomechanical properties of the cornea in a population-based study of elderly subjects with a long history of exposure to these factors.

MATERIALS AND METHODS

The ALIENOR (Antioxydants, Lipides Essentiels, Nutrition, and Maladies OculaiRes) study is a prospective population-based epidemiologic study on age-related eye diseases performed at the University Hospital of Bordeaux (France). The complete methodology of this study was published previously.[19,20]

Study Population

The ALIENOR study aims at assessing the associations of age-related eye diseases with nutritional factors and takes into account other major determinants of eye diseases, including gene polymorphisms, environmental, and vascular factors.[19] Subjects of the ALIENOR study were recruited from an ongoing population-based study on the vascular risk factors for dementia, the Three-City (3C) Study.[20] The 3C Study included 9294 subjects aged 65 years or more from three French Cities (Bordeaux, Dijon, and Montpellier), 2104 of whom were randomly recruited in Bordeaux. Subjects were contacted individually from electoral rolls. They were initially recruited in 1999–2001 and followed up about every 2 years since that time. The ALIENOR study consists of eye examinations offered to all participants of the 3C cohort in Bordeaux since the third follow-up (2006–2008). Among the 1450 participants re-examined between October 2006 and May 2008, 963 (66.4%) participated in the ALIENOR study's baseline eye examination. This examination consisted of the evaluation of age-related eye diseases: AMD, glaucoma, cataract, and dry eye syndrome. Detailed characteristics of participants and nonparticipants have been published elsewhere.[19]

At the fourth follow-up (2009–2010), among the 963 participants, 624 (59 deaths, 265 refusals, 15 moving) participated in the ALIENOR study's second eye examination, which is the target of the present study. They were aged 74 years or more. An examination of biomechanical properties of the cornea using the ORA was included in the second eye examination.

For this study, we excluded participants with missing ORA measurements ($n = 30$) and those with a diagnosis of glaucoma ($n = 43$) or ocular hypertension ($n = 91$). Some participants had more than one exclusion criteria; the study sample was thus 497 participants.

This research followed the tenets of the Declaration of Helsinki. Participants gave written consent for the participation in the study. The design of the ALIENOR study was approved by the Ethical Committee of Bordeaux (Comité de Protection des Personnes Sud-Ouest et Outre-Mer III) in May 2006.

Eye Examination

Each subject underwent an ophthalmologic examination that included a best-corrected visual acuity measurement, an IOP measurement by a noncontact tonometer (KT 800; Kowa, Nagoya, Japan), macular and optic disc color photography by nonmydriatic retinophotograph (TRC NW6S; Topcon, Inc., Tokyo, Japan), and a spectral-domain optical coherence tomography examination of the macula and the optic nerve

head (Spectralis; Heidelberg Engineering, Heidelberg, Germany).

Central corneal thickness was measured using Pachpen (Accutome, Inc., Malvern, PA, USA), after local anesthesia with oxybuprocain eye drops. Pachpen is a hand-held ultrasound pachymeter showing high intraobserver repeatability and interobserver reproducibility and providing comparable corneal pachymetry than Scheimpflug technology or optical low-coherence reflectometry.[21,22] The use of eye drops was refused by 63 (12.7%) of the participants, because of previous allergy to eye drops or other contraindications. In addition, CCT measurements were missing in 61 (12.3 %) participants, mainly because of technical failure. Corneal thickness measurements were thus available in 373 subjects.

Corneal biomechanical properties were assessed using the ORA and software version 3.01. After checking for a good alignment of the eye and the probe, a series of four measurements was performed on both eyes of each subject in a sitting position as recommended by Reichert laboratories, and we studied the mean value of this series of four measurements. Corneal hysteresis (CH) and corneal resistance factor (CRF) are both viscoelastic parameters of the cornea obtained with the machine. Corneal hysteresis is defined as the difference between the loading applanation pressure (inward applanation) and the unloading applanation pressure (outward applanation) applied at the apex of the cornea by a calibrated air-puff and represents the viscoelastic response of the cornea. Corneal resistance factor is calculated as a linear function of both applanation pressures and is supposed to be more correlated with CCT.[23]

Clinical and Lifestyle Determinants

Socio-demographic, lifestyle, and medical history data were collected during a face-to-face interview using a standardized questionnaire administered by a trained psychologist or nurse. They included age, sex, educational level, and smoking status (never smokers, current smokers, and former smokers groups).

Body mass index (BMI; in kg/m^2) was calculated as weight/height^2 using measured weight and height. Plasma glucose and lipids were determined from fasting blood samples performed in 2011. Diabetes was defined as fasting blood glucose ≥ 7.0 mM, self-reported diabetes, or current use of antidiabetic treatment. Plasma lipids were categorized using the National Cholesterol Education Program-Adult Treatmen PanelIII (NCEP_ATPIII) classification.[24]

Lifetime Ambient UV Radiation Exposure

The methodology was previously published by Delcourt et al.[25] For each participant, average annual ambient UV radiation exposure was estimated using the residential history, by weighing annual ambient UV radiation at each location by the time spent at that location. Residential history from birth (locations, time spent at each location) was self-declared up to the first eye examination (2006–2008). In France, locations were divided in 101 geographical areas, corresponding to the 101 administrative departments (95 in metropolitan France and 6 overseas departments). Concerning foreign countries, ambient UV radiation was generally estimated for the capital of the country, except when the capital was very off-centered, in which case a more central location was chosen. Very large countries (United States, China, etc.) were excluded from this analysis, because an estimation of solar radiation in a single geographic location is meaningless.

Average annual ambient solar radiation was assessed for the first 65 years of life, because almost all subjects (97.3 %) lived in the Bordeaux area beyond this age. It was categorized into

three groups (lower quartile, intermediate quartile, and upper quartile). The intermediate quartiles corresponded to the subjects having lived all their lives in the Bordeaux area (latitude: 44°50′16″ north/longitude: 0°34′46″ west) and was used as the reference.

Ultraviolet Radiation. In each location, UV radiation was extracted from Eurosun UV database (www.eurosun-project.org). Briefly, UV irradiation levels were initially extracted from surface solar irradiance derived from the Meteosat satellite's images. From these irradiation levels, the UV component was computed by a model, which exploits the algorithm set up by the Royal Institute of Meteorology, Belgium, published in the European Solar Radiation Atlas (ESRA; spectral model of Joukoff ESRA). The algorithm converts the total irradiance (E) into its spectral distribution E (λ), every 10 nm, and gives estimates for total UV (280–400 nm), UVA (315–400 nm), and UVB (280–315 nm). The calculation of individual exposure assumes that the irradiation levels in different regions remained constant over the years. These estimates were available for European and North African countries, with a resolution of 5 km. However, they are not available for other countries (Americas, sub-Saharan Africa, Asia, Oceania).

Estimation of Missing UV Radiation Data Using Global Solar Radiation. In each location, global ambient annual solar radiation (a measure of solar energy including all wavelengths) was estimated using astronomical equations and the statistics of sunshine hours, using the same methodology as in the Pathologies Oculaires Liees a l'Age (POLA) Study.[26] Overall, global ambient annual solar radiation estimates were available in 116 locations (101 French departments, 7 European countries, 3 North African countries, and 5 other countries). In 105 locations (95 French departments, 7 European countries, and 3 North African countries), both UV radiation and global solar radiation (GSR) estimates were available. Pearson's correlation coefficient between these two variables was 0.952. For the 11 areas with missing UV radiation but available solar radiation (6 French overseas departments and 5 countries), we thus estimated ambient UV radiation from GSR, using linear regression modeling. The regression equation derived from the 105 locations with both UV radiation (UVR) and GSR was as follows: UVR $= 5613.105 + 0.0729 \times$ GSR ($r^2 = 0.91$). The same analyses were performed for UVA and UVB, leading to the following equations: UVA $= 5467.398 + 0.0710 \times$ GSR and UVB $= 145.708 + 0.00189 \times$ GSR.

Finally, estimates of UV radiation exposure could still not be estimated for some countries. When, for a given subject, the number of years spent in such countries was less than or equal to 3 years, these countries were eliminated from the calculations. For 71 subjects, average ambient UV radiation was therefore calculated on 62, 63, or 64 years instead of 65 years. In addition, 113 subjects were excluded from the analyses because they spent more than 3 years in countries where UV radiation could not be estimated.

Statistical Analysis

Variations of corneal parameters according to clinical and lifestyle determinants were performed using multivariate mixed linear regression, allowing one to take into account data from both eyes and their intraindividual correlation. We first performed age-, sex-, and IOP-adjusted models, using the corneal parameter as the dependent variable and the determinant of interest, age, sex, and IOP, as the independent variables. Second, for each corneal parameter, we performed multivariate models by selecting all variables associated with the corneal parameter, in addition to age, sex, and IOP ($P < 0.20$). All statistical analyses were performed using statistical software (SAS, version 9.2; SAS Institute, Inc., Cary, NC, USA).

RESULTS

Population Characteristics

The mean age was 82.2 ± 4.3 years (range, 75.6–96.6 years; median age, 81.4 years), and 63.0% ($n = 313$) of subjects were women. Mean CH was 9.4 ± 1.9 mm Hg, and mean CRF was 9.8 ± 1.9 mm Hg. Mean CCT was 551.6 ± 36.8 μm, and mean IOP was 16.3 ± 3.6 mm Hg.

Corneal Characteristics by Demographic and Lifestyle Risk Factors

In Table 1, corneal hysteresis and CRF values were significantly lower in older patients (sex- and IOP-adjusted difference with 95% of confidence interval [CI]: −0.43 [−0.72;−0.15] mm Hg; $P = 0.003$; and −0.37 [95% CI: −0.61;−0.12] mm Hg; $P = 0.004$, respectively), whereas CCT mean values were not significantly different. As the range of age was quite large, a cutoff age value of 80 years, close to the median age, was chosen for comparison of younger and older subjects. One hundred eighty-nine subjects were younger than 80 years of age, and 308 were 80 years of age or older.

We did not observe any associations of CH or CRF parameters with sex or smoking.

After adjustment for age, sex, and IOP, the analysis of the association of lifetime ambient UV radiation exposure with corneal parameters showed no differences of CCT between subjects having low or high lifetime ambient UV radiation exposure than subjects with medium exposure (reference group). By contrast, CH and CRF mean values were significantly lower in subjects having higher lifetime ambient UV radiation exposure than subjects of the reference group [CH: −0.45; 95% CI: −0.81;−0.10; $P = 0.01$; and CRF: −0.40; 95% CI: −0.71;−0.10]; $P = 0.01$), whereas there was no difference of CH and CRF values between subjects having a lower ambient UV radiation exposure during residential history and subjects of the reference group (CH: −0.04; 95% CI: −0.39;0.31; $P = 0.82$; and CRF: −0.02; 95% CI: −0.33;0.27; $P = 0.86$).

Central corneal thickness was significantly higher in the group of former smokers than in the reference group of never smokers (+9.85; 95% CI: 1.26;18.68; $P = 0.03$) and was not significantly associated with the other studied parameters.

Corneal Characteristics by Metabolic Risk Factors

In Table 2, subjects with diabetes had higher CH and CRF values than those without diabetes (CH: 0.61; 95% CI: 0.20;1.02; $P = 0.003$; and CRF: 0.53; 95% CI: 0.18;0.88; $P = 0.003$), whereas CCT mean values were not significantly different according to diabetes status ($P = 0.17$). Consistently, subjects having fasting blood glucose values greater than or equal than 7.0 mM had significantly higher CH and CRF mean values compared with subjects having fasting blood glucose values lower than 6.1 mM ($P < 0.05$).

Subjects with plasma LDL cholesterol greater than or equal than 4.13 mM had a borderline significantly lower mean CH value than subjects with plasma LDL cholesterol lower than 3.35 mM (age-, sex-, and IOP-adjusted difference of CH: −0.40; 95% CI: −0.82;−0.004; $P = 0.048$). We observed the same trend for CRF without reaching significance (age-, sex-, and IOP-adjusted difference of CRF: −0.33; 95% CI: −0.67;0.02; $P = 0.06$).

After age, sex, and IOP adjustment, BMI, high plasma triglycerides, and low plasma HDL cholesterol were not significantly associated with CH, CRF, or CCT in our population sample.

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TABLE 1. Variation of Corneal Characteristics According to Demographic and Lifestyle Risk Factors

Characteristics	CCT (μm)				CH (mm Hg)				CRF (mm Hg)			
	N	Mean	Age-, Sex-, and IOP-Adjusted Difference (95% CI)	P Value	N	Mean	Age-, Sex-, and IOP-Adjusted Difference (95% CI)	P Value	N	Mean	Age-, Sex-, and IOP-Adjusted Difference (95% CI)	P Value
Age (y)												
<80	283	551.91	Reference		375	9.67	Reference		375	9.99	Reference	
≥80	460	551.38	−0.55 (−7.89;6.79)	0.88	611	9.23	**−0.43 (−0.72;−0.15)**	**0.003**	611	9.66	**−0.37 (−0.61;−0.12)**	**0.004**
Sex												
Male	289	555.02	Reference		364	9.31	Reference		364	9.74	Reference	
Female	454	549.39	−5.26 (−12.56;2.05)	0.16	622	9.45	0.13 (−0.16;0.42)	0.37	622	9.82	0.12 (−0.13;0.37)	0.34
IOP (mm Hg)			**0.97 (0.51;1.43)**	**<0.0001**			**−0.09 (−0.12;−0.05)**	**<0.0001**			**0.22 (0.19;0.25)**	**<0.0001**
Smoking												
Never smokers	477	548.18	Reference		642	9.41	Reference		642	9.72	Reference	
Former smokers	231	559.19	**9.85 (1.26;18.68)**	**0.03**	298	9.39	0.10 (−0.24;0.73)	0.56	298	9.89	0.11 (−0.19;0.41)	0.48
Current smokers	35	547.63	−2.41 (−19.50;14.68)	0.78	46	9.25	0.05 (−0.64;0.73)	0.90	46	10.14	0.10 (−0.49;0.68)	0.74
Mean annual ambient UV exposure (kJ/cm²)												
<39.649	185	550.68	−3.95 (−16.26;2.45)	0.40	238	9.46	−0.04 (−0.39;0.31)	0.82	238	9.86	−0.02 (−0.33;0.27)	0.86
39.649–40.173	303	553.39	Reference		421	9.52	Reference		421	9.88	Reference	
≥40.173	177	548.66	−6.90 (−14.73;1.26)	0.15	221	9.07	**−0.45 (−0.81;−0.10)**	**0.01**	221	9.44	**−0.40 (−0.71;−0.10)**	**0.01**

Results in bold are statistically significant ($P < 0.05$). *N*, number of eyes.

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TABLE 2. Variation of Corneal Characteristics According to Metabolic Risk Factors

Characteristics	CCT (μm)				CH (mm Hg)				CRF (mm Hg)			
	N	Mean	Age-, Sex-, and IOP-Adjusted Difference (95% CI)	P Value	N	Mean	Age-, Sex-, and IOP-Adjusted Difference (95% CI)	P Value	N	Mean	Age-, Sex-, and IOP-Adjusted Difference (95% CI)	P Value
Diabetes mellitus												
No	513	548.90	Reference		695	9.28	Reference		695	9.63	Reference	
Yes	109	558.17	7.32 (−3.04;17.67)	0.17	137	9.79	**0.61 (0.20;1.02)**	**0.003**	137	10.35	**0.53 (0.18;0.88)**	**0.003**
Fasting blood glucose (mM)												
<6.1	490	547.97	Reference		665	9.29	Reference		665	9.63	Reference	
6.1–7.0	60	559.40	8.87 (−4.59;22.32)	0.20	75	9.37	0.10 (−0.43;0.63)	0.71	75	9.89	0.09 (−0.37;0.54)	0.70
≥7.0	42	550.76	2.83 (−12.87;18.52)	0.72	56	9.80	**0.62 (0.02;1.23)**	**0.04**	56	10.41	**0.55 (0.03;1.07)**	**0.04**
BMI (kg/m^2)												
<25	350	550.41	Reference		458	9.39	Reference		458	9.75	Reference	
25–30	300	551.00	−0.43 (−8.17;7.31)	0.91	400	9.39	0.003 (−0.30;0.31)	0.99	400	9.80	0.002 (−0.26;0.27)	0.99
>30	89	558.63	6.03 (−5.81;17.92)	0.32	124	9.43	−0.01 (−0.47;0.45)	0.96	124	9.91	−0.01 (−0.40;0.38)	0.96
Plasma LDL cholesterol (mM)												
<3.35	275	551.44	Reference		365	9.41	Reference		365	9.78	Reference	
3.35–4.13	195	550.13	0.95 (−8.59;10.50)	0.84	245	9.43	−0.07 (−0.44;0.29)	0.66	245	9.75	−0.08 (−0.39;0.24)	0.59
≥4.13	132	545.73	−3.85 (−14.74;7.04)	0.49	196	9.05	**−0.40 (−0.82;−0.004)**	**0.048**	196	9.52	−0.33 (−0.67;0.02)	0.06
Plasma HDL cholesterol (mM)												
Normal	504	550.78	Reference		669	9.35	Reference		669	9.73	Reference	
Abnormal*	98	544.52	−7.14 (−17.86;3.59)	0.19	139	9.24	−0.09 (−0.50;0.31)	0.65	139	9.62	−0.08 (−0.43;0.26)	0.63
Plasma triglycerides (mM)												
<1.71	503	550.22	Reference		663	9.33	Reference		663	9.70	Reference	
≥1.71	99	547.41	−3.89 (−14.65;6.87)	0.48	145	9.32	−0.04 (−0.44;0.36)	0.83	145	9.77	−0.04 (−0.38;0.30)	0.83

Results in bold are statistically significant ($P < 0.05$).
* <1.03 mM in men and <1.29 mM in women.

TABLE 3. Multivariate Models of Determinants of Corneal Characteristics

Characteristics	CCT (μm)	*P* Value	CH (mm Hg)	*P* Value	CRF (mm Hg)	*P* Value
Age (y)						
<80	Reference		Reference		Reference	
≥80	−7.31 (−16.04;1.43)	0.10	**−0.56 (−0.89;−0.24)**	**0.0007**	**−0.48 (−0.75;−0.20)**	**0.0008**
IOP (mm Hg)	**0.90 (0.36;1.45)**	**0.001**	**−0.10 (−0.14;−0.06)**	**<0.0001**	**0.22 (0.18;0.25)**	**<0.0001**
Sex						
Male	Reference		Reference		Reference	
Female	−1.91 (−11.63;7.80)	0.70	0.19 (−0.15 ;0.54)	0.27	0.20 (−0.13 ;0.52)	0.23
Smoking						
Never smokers	Reference				Reference	
Former smokers	**11.01 (0.48;21.55)**	**0.04**			0.08 (−0.27;0.42)	0.66
Current smokers	−3.62 (−23.37;16.13)	0.72			−0.14 (−0.75;0.47)	0.65
Mean annual ambient UV exposure (kJ/cm²)						
<39.649	−5.75 (−16.32;4.82)	0.29	−0.06 (−0.44;0.32)	0.75	−0.06 (−0.39;0.27)	0.74
39.649–40.173	Reference		Reference		Reference	
≥40.173	−8.62 (−18.86;1.63)	0.10	**−0.50 (−0.88;−0.12)**	**0.01**	**−0.46 (−0.78;−0.13)**	**0.007**
Diabetes mellitus						
No	Reference		Reference		Reference	
Yes	2.68 (−9.85;15.21)	0.67	0.38 (−0.10;0.86)	0.12	0.34 (−0.07;0.75)	0.11
Plasma LDL cholesterol (mM)						
<3.35			Ref		Ref	
3.35–4.13			−0.13 (−0.51;0.25)	0.49	−0.14 (−0.47;0.18)	0.39
≥4.13			**−0.45 (−0.86;−0.03)**	**0.03**	**−0.37 (−0.72;−0.008)**	**0.04**
Plasma HDL cholesterol (mM)						
Normal	Reference					
Abnormal*	−6.46 (−18.03;5.10)	0.27				

Results in bold are statistically significant ($P < 0.05$).

* <1.03 mM in men and <1.29 mM in women.

Multivariate Analysis

In the multivariate analysis shown in Table 3, CH and CRF values remained significantly lower in subjects aged 80 years or older (CH- and CRF-adjusted difference: −0.56; 95% CI: −0.89;−0.24; $P < 0.001$; and −0.48; 95% CI: −0.75;−0.20; $P < 0.001$, respectively).

Intraocular pressure was still significantly associated with CCT (CCT-adjusted difference: 0.90; 95% CI: 0.36;1.45; $P = 0.001$) and ORA parameters (CH- and CRF-adjusted difference: −0.10; 95% CI: −0.14;−0.06; $P < 0.0001$; and 0.22; 95% CI: 0.18;0.25; $P < 0.0001$, respectively).

Subjects with lifetime ambient UV radiation exposure superior or equal to 40.173 kJ/cm² had lower CH and CRF mean values than subjects of the reference group (CH- and CRF-adjusted difference: −0.50; 95% CI: −0.88;−0.12; $P = 0.01$; and −0.46; 95% CI: −0.78;−0.13; $P = 0.007$, respectively). Subjects with lifetime ambient UV radiation exposure less than 39.649 kJ/cm² had no significant difference for CH and CRF values with the reference group (CH- and CRF-adjusted difference: −0.06; 95% CI: −0.44;0.32; $P = 0.75$; and −0.06; 95% CI: −0.39;0.27; $P = 0.74$, respectively).

In the multivariate analysis, neither CH- nor CRF-adjusted difference values remained significantly different in diabetics (CH: +0.38; 95% CI: −0.10;0.86; $P = 0.12$; and CRF: +0.34; 95% CI: −0.07;0.75; $P = 0.11$). However, subjects with plasma LDL cholesterol greater than or equal to 4.13 mM still had a significantly lower adjusted difference of the CH value compared with the reference group (CH: −0.45; 95% CI: −0.86;−0.03; $P = 0.03$), and the CRF value was significantly lower (CRF: −0.37; 95% CI: −0.72;−0.008; $P = 0.04$).

Finally, CCT was significantly higher in former smokers than in never smokers (+11.01; 95% CI: 0.48;21.55; $P = 0.04$) and was not significantly associated with age, sex, ambient UV radiation exposure, or metabolic factors in our population sample.

DISCUSSION

The current study is the first population-based study showing a modification of biomechanical properties of the cornea associated with age, lifetime ambient UV radiation exposure, and some metabolic factors in a large and unselected elderly population sample. Narayanaswamy et al.[27] reported a significant negative correlation between CH and CRF parameters and age in a younger cohort of a Chinese population. Some case-control studies confirmed these findings, with lower CH and CRF values with increasing age.[28-30] We observed lower CH and CRF values in the group of older subjects, and mean CH and CRF values of our population are lower than those observed in the literature, performed in younger subjects. As the mean age of our cohort was 82.2 years, our results confirmed that CH and CRF values continue to decrease with increasing age. On a stress-strain analysis of human corneas, Elsheikh et al.[31] demonstrated an increase in stiffness of approximately 11%–16% per decade associated with an increase in elasticity Young's modulus with age. Furthermore, Daxer et al.[32] showed an increase in collagen fibril diameter and intermolecular Bragg spacing with increasing age, whereas Malik et al.[33] observed an expansion of intermolecular spacing related to the accumulation of glycation end products, a crosslinking of molecules, and changes of the interfibrillar

matrix composition associated with age. Thus, these ultra-structural changes may explain the continuous viscoelastic change of the cornea associated with increasing age and the decrease in CH and CRF values that was observed. We did not observe any association between sex and corneal parameters in our population sample. This viscoelastic change is more likely related to the high mean age of our population sample and an expected lower effect of sex hormones on the corneal tissue, especially in the female group.[34,35]

To our knowledge, the effect of ambient UV radiation exposure during a lifetime on biomechanical properties of the cornea has never been evaluated. We observed a decrease in CH and CRF values for subjects with a higher ambient UV radiation exposure over a 65-year period of time, suggesting a viscoelastic change of the cornea, whereas it had no effect on CCT values. Interestingly, the biomechanical behavior of such corneas is comparable to the biomechanical behavior observed with increasing age. The effect of chronic UV radiation exposure on premature skin aging, defined as photoaging, has been widely described in the literature.[36,37] Ultraviolet-irradiated aged skin had more severe impairment of elastin and collagen fibrillar organization than chronologically aged skin, and the pathophysiologic process of chronic UV exposure leading to skin damage was close to the aging process.[38] Ultraviolet irradiation promotes oxidative stress, induces an increased production of metalloproteinases (MMP-1, MMP-3, and MMP-9), and activates signal transduction, leading to the production of cytokines and growth factors in skin connective tissue. Thus, the type 1 and 3 mature fibrillar collagen bundle breakdown process is stimulated, and the synthesis of the new collagen fibrils with its triple helix configuration is reduced and altered by the excessive action of metalloproteinases.[39–42] Cornea, mainly composed of type 1 fibrillar collagen bundles, absorbs a significant portion of UVB radiation (range of wavelength: 280–330 nm), particularly in the corneal epithelium, Bowman's layer, and anterior layers of the stroma.[1,43] Additionally, UVB has been shown to induce the production of MMP-2 and MMP-9 by the corneal epithelium and stroma and to initiate proteolytic activity in the cornea, leading to an increased crosslinking of the fibrillar collagen bundles.[44,45] All these findings may explain the changes of biomechanical behavior of the cornea we observed in subjects having a high ambient UV exposure during their lifetime, with a likely photoaging process of the cornea.

Although tobacco is a frequent environmental risk factor in the general population, its influence on the biomechanical properties of the cornea has scarcely been analyzed. In a case-control study, Hafezi et al.[46] showed higher CH and CRF values in smokers than in never smokers and did not report any comparison of CCT mean values between both groups. In the multivariate analysis, we did not find any significant differences of CH and CRF parameters between current or former smokers and never smokers, but CCT was significantly higher in former smokers than in the never smokers group. As in published studies, CH and CRF values are strongly and positively correlated with CCT values; tobacco smoking may influence biomechanical properties measurements by increasing CCT and thus overestimating CH and CRF values.[9,23,30]

In analyses adjusted for age, sex, and IOP, diabetes was associated with higher CH and CRF values. However, this difference was no longer significant after multivariate adjustment. This finding could be mainly explained by a relatively small sample size of diabetic patients in our population compared with published case-control studies. Some studies observed similar trends with statistical significance and suggested an increased dampening effect and increased viscoelasticity of such corneas.[27,47,48] Scheler et al.[48] stated that the increased dampening effect was most likely due to the

glycosylation of proteoglycans and glycosaminoglycans of the corneal extracellular matrix. Additionally, corneal collagen is modified in diabetic patients, and the accumulation of advanced glycation end products on basement membrane, especially laminin, can play a role in the corneal biomechanic modification of diabetic subjects.[49,50] These ultrastructural changes may modify the biomechanical properties of the cornea and affect IOP measurements. Indeed, some studies have demonstrated that IOP measurements using tonometry may be overestimated in diabetic patients, whereas diabetes may be associated with a lower risk of primary open angle glaucoma.[10-12,14] Although the exact association between diabetes and glaucoma onset or IOP measurements is unclear, diabetic ultrastructural changes of the cornea might partially influence its biomechanical properties. Interestingly, our finding could also be explained by a confounding effect of plasma LDL cholesterol. Indeed, when running the multivariate model after excluding only plasma LDL cholesterol, the associations with diabetes were much stronger (CH: 0.51; 95% CI: 0.09;0.93; $P = 0.02$; and CRF: 0.44; 95% CI: 0.08;0.79; $P = 0.02$) without modifying other age-, sex-, and IOP-adjusted associations.

The current study also showed an association of elevated plasma LDL cholesterol with biomechanical properties of the cornea, after adjustment for age, sex, and IOP. To our knowledge, this association has never been reported. Gaynor et al.[51] demonstrated a progressive extracellular accumulation of lipoprotein particles with age between corneal collagen bundles. These particles are predominantly made of cholesteryl ester spherical lipoproteins, and the deposition was mainly observed in the peripheral cornea where limbal vessels are located, but was also observed in the central cornea. The progressive deposit of lipoprotein particles between corneal collagen bundles with age and their progressive repartition between central and peripheral cornea could alter the global dampening effect of the cornea under air-puff pressure and could explain the viscoelastic change observed in our population sample.

The ultrastructure of the cornea seems to progressively change during the lifetime under the influence of age-related physiologic conditions and some frequent metabolic or environmental factors. As viscoelastic properties of the cornea result from an interaction between fibrillar collagen bundles and the extracellular matrix, the dampening of the cornea measured with the ORA could be continuously affected by these factors and could explain all our findings.

Population-based studies allow a better analysis of the influence of environmental and metabolic factors on biomechanical properties of the cornea compared with selected case-control studies by avoiding selection biases of the population sample. Furthermore, our study sample was composed of a large and unselected Caucasian population, and its original feature is represented by a very high mean age (82.2 years) of subjects with a longer history of exposure to the main metabolic or environmental factors observed during their lifetime. Thus, this study design allowed us to accurately analyze the influence of these factors on biomechanical properties of the cornea for subjects of the same ethnicity to better understand IOP measurement readings and the onset or progression of diseases such as primary open angle glaucoma and keratoconus. We also identified some limitations in our study when analyzing this population sample. First, we could have selective survival biases related to the elevated mean age of our population sample, which tends to be healthier than the general population. For instance, the influence of smoking on biomechanical properties of the cornea could not be accurately analyzed because of a low rate of current smoker subjects in our study population related to

a lower life expectancy of this subgroup of the population. Hence, our findings need further confirmation in a larger group of current smokers. Second, despite the accuracy of the Eurosun database and residential history questionnaires, some subjects were excluded from the analysis because of unavailable data outside Europe and North Africa, thus limiting the statistical significance of our findings. Moreover, only estimates of ambient UV radiation exposure were available, based on residential history and satellite-based estimates of UV radiation, whereas no data were available on sun-related behaviors, skin pigmentation, or sun sensitivity. This may have led to significant misclassification of exposures, because actual ocular UV radiation exposure may be quite different from ambient UV radiation exposure in those subjects with very low time spent outdoors. However, it seems most likely that misclassification was unrelated to ambient UV radiation (i.e., that there would be similar proportions of people with low/high outdoors activities in areas with different ambient UV radiation) and was thus unlikely to have biased the associations of biomechanical properties of the cornea with UV radiation exposure. Additionally, despite some limitations to accurately estimate lifetime UV radiation exposure for each subject, the use of satellite-based estimates probably improves its reliability by taking into account weather conditions in addition to latitude and altitude parameters at each location. Finally, when using the same methodology, the well-known association of UV radiation exposure with cataract was confirmed in our study and in the POLA study, suggesting that our measure of UV radiation exposure had some validity.[25,26]

In summary, we found biomechanical properties of the cornea measured by the ORA to be associated with increasing age, IOP, elevated plasma LDL cholesterol, and higher lifetime UV radiation exposure. It confirms results of published studies with regard to age and provides new insights on the potential influence of lifetime chronic UV radiation exposure on the photoaging process of the cornea and modified corneal biomechanics. The role played by all these different determinants of corneal biomechanics and, particularly, the manner they influence the onset and progression of ocular diseases such as glaucoma, keratoconus, or postoperative corneal ectasia, as well as IOP measurements, need further investigation to be confirmed and quantified.

Acknowledgments

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