Laser Scanning Tomography in the EPIC-Norfolk Eye Study: Principal Components and Associations

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Purpose. To describe Heidelberg Retina Tomograph (HRT) measures, their principal components, and their associations in a British population.

Methods. The European Prospective Investigation of Cancer (EPIC)-Norfolk Eye Study is nested within a multicenter cohort study. Measurements were taken with the HRT-2 and the software subsequently updated to yield HRT-3 parameters. Principal components analysis (PCA) was used to identify distinct components of the HRT variables. Generalized estimating equation models were used to examine associations of these components with age, sex, height, body mass index (BMI), blood pressure, social class, education, alcohol intake, smoking status, axial length, IOP, and lens status.

Results. Complete data were available from 10,859 eyes of 6430 participants with a mean age of 68 years. Principal components analysis identified three components with an eigenvalue greater than 1, explaining 79.9% of the variance of all the HRT measures. These were named *cup*, *retinal nerve fiber layer* (RNFL), and *rim* based on the factor loadings they were most correlated with. Older age was significantly associated with a greater *cup* ($P = 0.003$), smaller *RNFL* ($P < 0.001$), and smaller *rim* ($P < 0.001$). Female sex ($P = 0.001$), higher education ($P < 0.001$), and shorter axial length ($P < 0.001$) were associated with a greater *RNFL*. Lower BMI and higher IOP were associated with a greater *cup* (both, $P < 0.001$) and a smaller *rim* (BMI, $P = 0.001$; IOP, $P < 0.001$).

Conclusions. Heidelberg Retina Tomograph measures in this cohort were largely explained by three principal components related to optic disc *cup*, *RNFL*, and *rim*. Associations with *cup* and *rim* were distinct to associations with *RNFL*, suggesting different underlying determinants.

Keywords: diagnostic techniques, epidemiology, axial length, glaucoma, body mass index, optic disk

Structural assessment of the optic disc is an important component of the workup of patients with suspected or established glaucoma.[1,2] Optic disc photography is a common, low-cost method of assessment, but may suffer from high intra- and interobserver variability for the diagnosis of glaucoma.[3] Newer imaging devices provide quantitative structural measures that may be useful for the diagnosis of glaucoma and detection of disease progression.[4,5] The Heidelberg Retina Tomograph (HRT) is one such device which creates reproducible 3-dimensional (3D) images of the optic nerve head using scanning laser ophthalmoscopy.[6,7]

While some risk factors for POAG are well established (such as age, race, family history, and raised IOP),[8] the evidence for others such as sex,[9] and anthropometric measures[10] is inconsistent. This may be a result of small effects not being detected due to misclassification of glaucoma as a dichotomous outcome. Certainly, the reported prevalence of glaucoma varies greatly depending on the definition used.[11] Objective quantitative measures related to glaucoma, such as HRT-derived parameters, may provide greater power for detecting weak associations. This "endophenotype" approach has been fruitful in genetic association studies for glaucoma.[12] Understanding what influences optic nerve head structural measures at a population level may provide insight into the aetiology of glaucoma.

The aims of this study were to:

1. Describe HRT-derived optic nerve head measures in a predominantly White British population;
2. Examine the correlation structure of the glaucoma-related HRT measures to determine their principal components; and
3. Describe systemic and ocular associations with these HRT component measures.

METHODS

Participants

The European Prospective Investigation into Cancer (EPIC) study is a pan-European prospective population-based cohort study.[13] EPIC-Norfolk , one of the United Kingdom (UK) arms of EPIC, recruited and examined 25,639 residents aged 40 to 79 years between 1993 and 1997 for the baseline examination.[14] Recruitment was via general practices in the city of Norwich and the surrounding small towns and rural areas, and methods have been described in detail previously.[14] Since virtually all residents in the UK are registered with a general practitioner through the National Health Service, general practice lists serve as population registers. Ophthalmic assessment formed part of the third health examination and this is termed the EPIC-Norfolk Eye Study.[15] In total, 8623 participants were seen for the ophthalmic examination, between 2004 and 2011. The EPIC-Norfolk Eye Study was carried out following the principles of the Declaration of Helsinki and the Research Governance Framework for Health and Social Care. The study was approved by the Norfolk Local Research ethics committee (05/Q0101/191) and the East Norfolk & Waveney National Health Science Research Governance Committee (2005EC07L). All participants gave written, informed consent.

Measurements

Scanning laser ophthalmoscopy of each eye was carried out using the HRT 2 (Heidelberg Engineering, Heidelberg, Germany) following entering the participant's keratometry and refraction (Auto-Refractor 500; Humphrey Instruments, San Leandro, California). If image quality was poor (topography SD > 40 μm) a repeat scan was undertaken. Contours around the disc margins were manually drawn and subsequently checked by an ophthalmologist (and redrawn if necessary). The HRT software was subsequently updated to Glaucoma Module Premium Edition (software version 3.1; Heidelberg Engineering) and data exported following this. This derived data that is equivalent to HRT-3–derived parameters.

Axial length was measured using a Zeiss IOLMaster Optical Biometer (Carl Zeiss Meditec Ltd., Welwyn Garden City, UK). Five measurements were taken per eye and a mean was calculated. Intraocular pressure was measured using a non-contact appliance, the Ocular Response Analyzer (ORA; Reichert, Corp., Buffalo, NY). Three readings were taken per eye and the single best value of the Goldmann-correlated value used (based on the best quality pressure waveform as assessed by the ORA software). All ocular examinations were carried out without pupil dilation.

Height and weight were measured at the third health examination, with participants wearing light clothing and no shoes. Height was measured to 0.1 cm using a stadiometer, and weight was measured to the nearest 0.1 kg using digital scales (Tanita UK Ltd., Middlesex, UK). Body mass index (BMI) was calculated as weight/height.[2] Self-reported alcohol intake and smoking status were also ascertained at the third health examination. Alcohol intake was calculated as units consumed per week based on a questionnaire asking how much of beer/cider/lager (half pints), wine (glasses), sherry/fortified wine (glasses), or spirits (single measures) were drunk for each day in the last week. Blood pressure was also measured in the third health examination using an objective measurement device (Accutorr Plus; Datascope Patient Monitoring, Mindray UK, Ltd., Huntington, UK). Social class and educational level were ascertained at the first health examination. Social class was recorded according to the Registrar-General's occupation-based classification system and was based on the participant's last occupation if they were retired. Educational level was recorded and classified into four groups according to the highest qualification achieved.

Statistical Analysis

Description of HRT Parameters and Associations With Disc Area. For *t*-test comparisons of HRT parameters between men and women, we considered the mean value of both eyes for each participant. For all subsequent analyses, we considered data from both eyes of each participant and used generalized estimating equation models to account for the correlation between eyes. Univariable linear regression models were used to examine associations of optic disc area (outcome variable) with age, sex, height, BMI, systolic and diastolic blood pressure, social class, educational level, alcohol intake, smoking status, axial length, IOP, and lens status (explanatory variables). All parameters significant at the *P* less than 0.1 level were then included together in one multivariable linear regression model to examine independent associations with disc area.

Principal Components Analysis. Correlations between HRT variables were examined by the construction of a correlation matrix. Principal components analysis (PCA) was used to identify distinct components of all HRT variables, except for disc area. We did not include disc area in the PCA as our aim was to identify components of HRT variables that change with progressive glaucomatous damage and that describe the spectrum of health of an optic disc. However, when examining associations with the HRT components in subsequent analyses, we adjusted for disc area given the correlation with several HRT parameters (see below). Components with an eigenvalue greater than 1 after varimax rotation of the factor loadings were retained. An eigenvalue quantifies the amount of variance explained by a component, and a threshold of greater than 1 is commonly used to select principal components as this equates to each component explaining at least as much information as one original variable. The components were named according to the HRT variables they were most strongly correlated with (there were 3 components, which were named *cup*, *retinal nerve fiber layer* [*RNFL*] and *rim* – see Results section).

Associations With Components. Linear regression models were used to examine crude associations between the three components (outcome variables) and age, sex, height, BMI, systolic and diastolic blood pressure, social class, educational level, alcohol intake, smoking status, axial length, IOP, and lens status (explanatory variables). All models were adjusted for disc area given that rim area[16-23] and cup-to-disc ratio[23-26] are consistently positively associated with optic disc area. All parameters significant at the *P* less than 0.1 level for any of the components were then included together in three multivariable linear regression models, one for each component. Again, these models were all adjusted for disc area. While the HRT algorithm makes use of keratometry and refraction information, this may not account for ocular magnification completely. Axial length has been shown to be more closely related to magnification than keratometry.[27] Magnification correction for axial length is linear. We also repeated analyses further adjusted for the square of axial length for area parameters, such as disc area. To further examine the association between sex and *RNFL*, we repeated the analysis stratified by tertiles of age. Since the PCA-derived components had no specific units or scale that were of clinical significance, three further multivariable linear regression models were constructed replacing the PCA component outcome variable with a HRT parameter that was familiar clinically and well correlated with the component

TABLE 1. HRT Variables by Sex

	Men $n = 2825$	Women $n = 3605$	P Value
Disc area, mm^2	1.897 (0.418)	1.832 (0.400)	<0.001
Cup area, mm^2	0.478 (0.349)	0.433 (0.328)	<0.001
Rim area, mm^2	1.420 (0.326)	1.399 (0.324)	0.013
Cup-to-disc area ratio	0.238 (0.144)	0.224 (0.141)	<0.001
Rim-to-disc area ratio	0.762 (0.144)	0.776 (0.141)	<0.001
Cup volume, mm^3	0.102 (0.113)	0.087 (0.100)	<0.001
Rim volume, mm^3	0.345 (0.129)	0.354 (0.132)	0.007
Mean cup depth, mm	0.191 (0.089)	0.183 (0.086)	0.001
Maximum cup depth, mm	0.516 (0.209)	0.497 (0.204)	<0.001
Height variation contour, mm	0.357 (0.098)	0.364 (0.091)	0.003
Cup shape measure	−0.175 (0.062)	−0.175 (0.063)	1.00
Mean RNFL thickness, mm	0.215 (0.063)	0.225 (0.065)	<0.001
RNFL cross-sectional area, mm^2	1.040 (0.305)	1.069 (0.315)	<0.001
Horizontal cup-to-disc ratio	0.448 (0.208)	0.425 (0.204)	<0.001
Vertical cup-to-disc ratio	0.358 (0.226)	0.329 (0.228)	<0.001
CLM temporal–superior, mm	0.169 (0.067)	0.178 (0.068)	<0.001
CLM temporal–inferior, mm	0.141 (0.072)	0.153 (0.072)	<0.001
Linear cup-to-disc ratio	0.453 (0.173)	0.437 (0.173)	<0.001

Numbers presented are means (SD). *P* values were derived from *t*-tests comparing means in men and women. CLM, contour line modulation.

TABLE 2. Adjusted Associations With Optic Disc Area

	β	95% CI	P Value
Age, per decade	−0.011	(−0.025, 0.003)	0.14
Sex			
Male	Ref		
Female	−0.007	(−0.037, 0.022)	0.62
Height, per 10 cm	0.027	(0.011, 0.043)	0.001
DBP, per 10 mm Hg	0.009	(−0.002, 0.020)	0.10
Education level			
Degree	Ref		
A level	0.014	(−0.013, 0.042)	0.31
O level	−0.014	(−0.050, 0.023)	0.47
Less than O level	0.012	(−0.020, 0.043)	0.46
Alcohol intake			
No intake	Ref		
> 0 < 7 units/wk	−0.010	(−0.038, 0.017)	0.46
≥ 7 < 14 units/wk	0.022	(−0.007, 0.051)	0.14
≥ 14 < 21 units/wk	0.019	(−0.016, 0.055)	0.29
≥ 21 units/wk	−0.010	(−0.041, 0.021)	0.53
Axial length, mm	0.038	(0.030, 0.047)	<0.001
IOP, mm Hg	0.002	(0.000, 0.004)	0.039
Lens status			
Phakic	Ref		
Pseudophakic	−0.016	(−0.050, 0.018)	0.35

Results are from one multiple linear regression model with all variables presented in the model. *P* values <0.01 are in bold. O and A levels are standard educational examinations taken at 16 and 18 years of age in the UK. DBP, diastolic blood pressure. Ref, reference level.

(linear cup-to-disc ratio, mean RNFL thickness, and rim area). These three regression analyses were then repeated in participants not reporting a history of glaucoma medication use or a glaucoma procedure. Stata version 12.1 (StataCorp LP, College Station, TX) was used for all analyses.

RESULTS

In total, 15,694 eyes of 8064 participants underwent examination with the HRT, of which 11,946 eyes of 7009 participants had good quality scans (topography SD ≤ 40). Of these, there were complete data for all covariables from 10,859 eyes of 6430 participants and the main analyses refer to these eyes. Compared with excluded participants (those attending the EPIC-Norfolk Eye Study but without a good quality HRT image or complete data for covariables, *n* = 2193), included participants were of similar weight, BMI, social class, and educational level, but were significantly younger (*P* < 0.001) and more were female (*P* = 0.007). Included participants had a mean age of 68 years (range 48–90) and 56% were women.

Mean values of global HRT parameters for the cohort, stratified by sex, are presented in Table 1. These are crude values and not adjusted for height. There were intersex differences for all parameters except cup shape measure. On average, men had larger disc area and cup-related measures. Women had larger rim-related measures (except rim area) and RNFL-related measures.

Univariable associations with optic disc area are presented in Supplementary Table SA. In the multivariable analysis (Table 2), only height and axial length were significantly associated with disc area (0.027 mm^2 per 10 cm taller height, *P* = 0.001; and 0.038 mm^2 per millimeter longer axial length, *P* < 0.001).

Age, sex, and IOP were only significant at the 1% level in the univariable models.

Many HRT variables were strongly correlated with other HRT variables (Supplementary Table SB, Supplementary Fig. SA). Exploratory PCA identified three principal components with an eigenvalue greater than 1, explaining 79.9% of the variance of the data. Examination of the factor loadings of these three components (Table 3) found the first component to be correlated most with cup (and cup-disc ratio) related measures; the second component with disc margin RNFL related measures; and the third component with rim related measures. The components were therefore named *cup*, *RNFL*, and *rim*. Summary statistics and the correlation structure for the three components are summarized in Supplementary Tables SC and SD. A larger (more positive) *cup* or *rim* component measure represents a structurally larger cup or rim. A larger (more positive) *RNFL* component represents a thicker RNFL or more RNFL modulation at the disc margin.

Crude associations with the three components (adjusted for disc area only) are presented in Supplementary Table SE. Table 4 presents results from multivariable models for the three components. Similar associations were found for the *cup* and *rim* components, which were both different to associations found with the *RNFL* component. Older age was significantly associated with all three components. Women had a significantly larger *RNFL* component, but there were no significant sex differences for *cup* or *rim*. Similarly, higher educational level and shorter axial length were associated with a larger *RNFL* component, but there were no significant associations with *cup* or *rim*. Conversely, higher BMI was associated with a smaller *cup* component and a larger *rim* component, but there was no significant association with *RNFL*. Higher IOP was associated with a larger *cup* and smaller *rim*, but there was no significant association with *RNFL*. There appeared to be more significant associations between height and lens status with

TABLE 3. Results from Principal Components Analysis

	Component 1 *Cup*	Component 2 *RNFL*	Component 3 *Rim*
Cup area, mm^2	**0.364**	−0.078	0.178
Rim area, mm^2	0.012	−0.060	**0.777**
Cup-to-disc area ratio	**0.336**	−0.045	−0.039
Rim-to-disc area ratio	**−0.336**	0.045	0.039
Cup volume, mm^3	**0.338**	−0.034	0.129
Rim volume, mm^3	−0.051	0.221	**0.506**
Mean cup depth, mm	**0.327**	0.164	−0.041
Maximum cup depth, mm	**0.283**	0.210	−0.108
Height variation contour, mm	0.007	**0.382**	−0.049
Cup shape measure	0.192	−0.063	0.126
Mean RNFL thickness, mm	−0.055	**0.482**	−0.098
RNFL cross-sectional area, mm^2	0.050	**0.442**	0.176
Horizontal cup-to-disc ratio	**0.304**	0.003	−0.076
Vertical cup-to-disc ratio	**0.316**	−0.024	−0.026
CLM temporal–superior, mm	0.001	**0.402**	−0.046
CLM temporal–inferior, mm	−0.027	**0.355**	−0.023
Linear cup-to-disc ratio	**0.321**	−0.006	−0.090

Following rotation, three components with an eigenvalue >1 explained 79.9% of the variance. The factor loadings are presented below, and based on these, the components were named cup, RNFL, and rim. Factor loading magnitudes >0.25 are in bold.

RNFL than *cup* and *rim* components. The multivariable associations presented in Table 4 did not change significantly after further adjustment for the square of axial length. A comparison of the association between female sex and *RNFL* across tertiles of age is shown in the Figure. The association was strongest in the youngest tertile (+0.308 [95% confidence interval (CI) 0.093, 0.524], $P = 0.005$), weaker in the middle tertile (+0.272 [0.060, 0.484], $P = 0.012$), and no longer significant in the oldest tertile (+0.118 [−0.108, 0.343], $P = 0.31$).

Given the arbitrary scale of the PCA-derived components, a clinically familiar representative measure was chosen for each component based on the factor loadings (Table 3) and frequency of use in clinical practice. Linear cup-to-disc ratio was chosen for *cup*, mean RNFL thickness for *RNFL*, and rim area for *rim*. Table 5 presents results from the multivariable analyses with the representative variables as the outcome variables. Similar patterns of association were found as with the principal components. Following exclusion of 203 participants with a history of glaucoma medication use ($n = 174$) or a glaucoma procedure ($n = 47$), repeated regression analyses revealed similar associations as for the full cohort (Supplementary Table SF). There were small reductions in magnitude of the coefficients for age, and pseudophakic lens status.

DISCUSSION

The HRT generates many correlated variables (Supplementary Table SB) and there is no consensus on which variables are most important clinically or for research when examining associations with optic disc structure. Principal components analysis is a useful technique in such situations as it can reduce

the data into fewer components. The process attempts to derive uncorrelated components, and therefore helps to identify distinct attributes of the data. We found 80% of the variance of the global HRT parameters to be explained by three components, which we named *cup*, *RNFL*, and *rim* according to the raw parameters with which they were most correlated. We therefore suggest, when assessing patients clinically or analyzing data in a research setting, examining at least one parameter from each category may be helpful. Linear cup-to-disc ratio, mean RNFL thickness and rim area yielded similar associations as the three component scores and these may be suitable variables (Table 5).

Interestingly, we found the *RNFL* component to have different systemic and ocular associations from the *cup* or *rim* components. Higher IOP and lower BMI were associated with larger *cup* and smaller *rim* components, but not associated with *RNFL*. Female sex, higher educational level, and shorter axial length were associated with a larger *RNFL* component, but not associated with *cup* or *rim* components. This contrasting epidemiology suggests there may be distinct determinants of optic disc anatomy and peripapillary RNFL anatomy, and is in keeping with current evidence regarding the association between IOP and optic nerve head structure. Raised IOP has been previously associated with optic disc parameters in population-based studies.[28-30] However, while hospital-based studies have reported thinner RNFL in ocular hypertensive patients compared with controls[31] and faster rates of RNFL thinning amongst glaucoma patients with higher IOP,[32] to the best of our knowledge, a significant association between IOP and RNFL measures has never been reported at a population level. A recent report from the Beijing Eye Study 2011 found no significant association between IOP and SD-OCT measured RNFL.[33] Furthermore, data from the Singapore Chinese Eye Study found IOP to be associated with SD-OCT measured rim area and vertical cup-to-disc ratio, but not average RNFL thickness.[34] Based on these findings, and the results of the present study, it may be hypothesized that there are different underlying mechanisms for optic disc cupping compared with RNFL thinning. For example, while RNFL thinning may mostly reflect retinal ganglion cell (RGC) loss, optic disc cupping may reflect both RGC loss and mechanical posterior deformation of the lamina cribrosa. In other words, IOP-induced stress and strain at the optic nerve head may induce some disc cupping independently of RGC loss, with minimal effect on RNFL thickness. Supporting this hypothesis is evidence of reduced disc cupping[35] and reversal of lamina cribrosa posterior displacement[36] following trabeculectomy in glaucoma patients.

We found higher BMI to be associated with a smaller optic disc cup and larger rim (both in a direction consistent with a reduced prevalence of POAG). A positive association between BMI and rim area has previously been reported,[37,38] as well as a protective effect of BMI on POAG.[10,39] Xu and colleagues postulated that the association between higher BMI and less optic disc cupping may be mediated by a higher cerebrospinal fluid pressure providing more lamina cribrosa support in opposition to IOP.[38] If BMI is mediating its effect via mechanical forces at the lamina cribrosa, the findings of an association of BMI with *cup* and *rim*, but not *RNFL*, would be in keeping with our hypothesis that mechanical changes at the optic nerve head may result in cupping that is partly independent of RGC loss, and with minimal change to RNFL thickness.

We also found associations between HRT parameters and age, sex and, axial length. We found older age to be independently associated with a larger cup, smaller rim, and thinner RNFL. While these associations were mildly reduced following exclusion of participants with a history of glaucoma

TABLE 4. Multivariable Associations With Component Scores

	Cup Component			RNFL Component			Rim Component		
	β	95% CI	*P* Value	β	95% CI	*P* Value	β	95% CI	*P* Value
Age, per decade	0.132	(0.046, 0.218)	**0.003**	−0.409	(−0.472, −0.345)	**<0.001**	−0.076	(−0.112, −0.039)	**<0.001**
Sex									
Male	Ref			Ref			Ref		
Female	−0.010	(−0.183, 0.163)	0.91	0.220	(0.094, 0.346)	**0.001**	0.021	(−0.05, 0.094)	0.58
Height, per 10 cm	0.124	(0.027, 0.221)	0.012	0.096	(0.026, 0.167)	**0.008**	−0.050	(−0.09, −0.009)	0.016
BMI, per 5 kg/m²	−0.133	(−0.202, −0.064)	**<0.001**	0.008	(−0.043, 0.058)	0.76	0.051	(0.021, 0.080)	**0.001**
SBP, per 10 mm Hg	−0.023	(−0.060, 0.013)	0.21	0.010	(−0.017, 0.036)	0.49	0.009	(−0.007, 0.024)	0.26
Education level									
Degree	Ref			Ref			Ref		
A level	0.144	(−0.022, 0.309)	0.09	−0.259	(−0.379, −0.138)	**<0.001**	−0.093	(−0.163, −0.023)	**0.009**
O Level	−0.030	(−0.247, 0.188)	0.79	−0.055	(−0.213, 0.103)	0.50	0.024	(−0.068, 0.116)	0.61
Less than O level	0.147	(−0.039, 0.333)	0.12	−0.293	(−0.429, −0.157)	**<0.001**	−0.071	(−0.150, 0.007)	0.08
Axial length, mm	0.011	(−0.040, 0.063)	0.66	−0.221	(−0.260, −0.182)	**<0.001**	−0.014	(−0.036, 0.009)	0.23
IOP, mm Hg	0.048	(0.035, 0.061)	**<0.001**	−0.002	(−0.012, 0.009)	0.73	−0.020	(−0.025, −0.014)	**<0.001**
Lens status									
Phakic	Ref			Ref			Ref		
Pseudophakic	0.210	(0.008, 0.412)	0.041	−0.313	(−0.474, −0.151)	**<0.001**	−0.106	(−0.194, −0.017)	0.019

Results are from three multiple linear regression models with the component score as dependent variable, and all explanatory variables presented included together in each model. *P* values <0.01 are in bold. SBP, systolic blood pressure.

therapy, it would appear that a change in these parameters occurs with age in healthy individuals, albeit at a likely slower rate. While there are several studies reporting a decline in RNFL thickness with age,[40-43] there is a surprising paucity of evidence for a decline in optic disc rim with age.[34,44,45] More studies have not reported a significant decline of rim area with age.[22,23,38,46] We found women to have a significantly larger *RNFL* component, consistent with a lower prevalence of POAG. The evidence for a sex predilection in POAG is inconsistent,[9] though our results may support the finding of a Bayesian meta-analysis, which suggested an increased risk in men.[47] Furthermore, our finding of a stronger sex effect in younger participants is consistent with results reported from the Melbourne Visual Impairment project, which showed
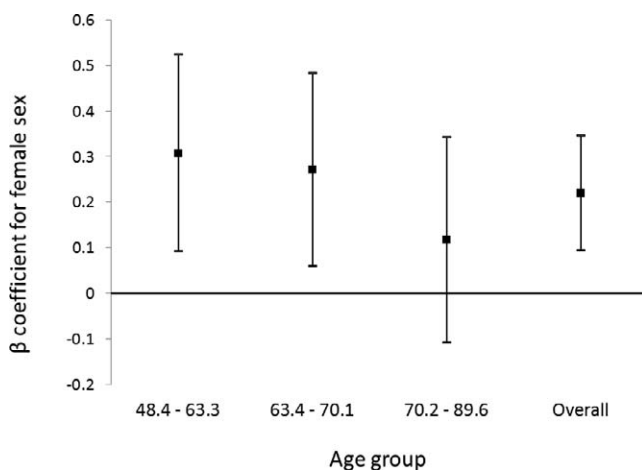

FIGURE. Beta coefficients with 95% CIs for the association of female sex with the RNFL component, stratified by tertiles of age, and for the whole cohort. The coefficients were derived from multivariable linear regression models adjusted for age, height, BMI, systolic blood pressure, education level, axial length, IOP, and lens status.

higher rates of incident open-angle glaucoma in men for all age groups except the oldest (80+).[48] This may be related to a protective effect of endogenous oestrogens in premenopausal women, consistent with the finding that earlier menopause may be a risk factor for glaucoma.[49,50] We found a longer axial length to be associated with a smaller *RNFL* component, but found no association with *cup* or *rim*. This lack of association with disc parameters is in contrast to several studies that have reported significant associations between rim parameters and axial length[29,46] or refraction.[30,51-53] However, there was no adjustment for disc area in any of these studies. In our population, the crude positive associations between axial length and *rim* or *cup* (data not shown) were explained by confounding by disc area. In other words, a longer axial length was not associated with a larger rim or cup over and above that expected with a larger disc area.

Heidelberg Retina Tomograph variables derived using software version 3 or later (as in the current study) have been shown to differ significantly from those derived using earlier software versions. Heidelberg Retina Tomograph-2–derived disc area, rim area, cup area, cup-disc area ratio, rim-disc area ratio, and rim volume were found to be systematically larger than HRT-3–derived equivalent variables in participants of the Singapore Malay Eye Study.[54] We found mean optic disc area to be 1.90 mm² and 1.83 mm² in men and women respectively; this is of similar magnitude to other published HRT-3–derived optic disc area measures, which have ranged from 1.77 mm² to 2.07 mm².[2,53-55] There is some evidence suggesting that larger optic disc area is a risk factor for POAG[56,57] and this has prompted work examining the determinants of disc area. Genetic polymorphisms in the *ATOH7* gene have been found to be associated with disc area (considered an endophenotype for POAG)[58-60] and this gene has subsequently been found to be associated with POAG.[61] We found significantly larger optic discs in participants who were taller and had longer axial length. Height was also significantly associated with disc area in the Rotterdam study,[51] although no association was found in other studies.[37,62-64] A significant, positive association between axial length and disc area was found in Chinese

TABLE 5. Multivariable Associations With HRT Variables Representative of Each Principle Component

	Linear Cup-to-Disc Ratio			Mean RNFL Thickness, mm			Rim Area, mm^2		
	β	95% CI	P Value	β	95% CI	P Value	β	95% CI	P Value
Age, per decade	0.010	(0.004, 0.015)	**0.001**	−0.015	(−0.017, −0.012)	**<0.001**	−0.022	(−0.031, −0.013)	**<0.001**
Sex									
Male	Ref			Ref			Ref		
Female	0.004	(−0.008, 0.015)	0.52	0.006	(0.002, 0.011)	**0.003**	0.000	(−0.019, 0.018)	0.97
Height, per 10 cm	0.009	(0.002, 0.015)	**0.008**	0.003	(0.000, 0.005)	0.036	−0.010	(−0.020, 0.000)	0.06
BMI, per 5 kg/m^2	−0.008	(−0.013, −0.004)	**<0.001**	0.001	(−0.001, 0.002)	0.37	0.013	(0.006, 0.020)	**<0.001**
SBP, per 10 mm Hg	−0.001	(−0.003, 0.001)	0.44	0.000	(−0.001, 0.001)	0.58	0.003	(−0.001, 0.007)	0.11
Education level									
Degree	Ref			Ref			Ref		
A level	0.010	(−0.001, 0.021)	0.06	−0.008	(−0.012, −0.004)	**<0.001**	−0.016	(−0.034, 0.001)	0.07
O Level	−0.003	(−0.017, 0.012)	0.71	−0.001	(−0.007, 0.004)	0.60	0.004	(−0.019, 0.027)	0.72
Less than O level	0.009	(−0.003, 0.021)	0.15	−0.009	(−0.014, −0.005)	**<0.001**	−0.014	(−0.034, 0.005)	0.15
Axial length, mm	0.002	(−0.001, 0.006)	0.18	−0.006	(−0.008, −0.005)	**<0.001**	−0.005	(−0.010, 0.001)	0.08
IOP, mm Hg	0.003	(0.002, 0.004)	**<0.001**	0.000	(−0.001, 0.000)	0.15	−0.005	(−0.006, −0.003)	**<0.001**
Lens status									
Phakic	Ref			Ref			Ref		
Pseudophakic	0.01s7	(0.003, 0.030)	0.016	−0.011	(−0.017, −0.006)	**<0.001**	−0.030	(−0.052, −0.008)	**0.007**

Results are from three multivariable linear regression models with the HRT variable as dependent variable, and all explanatory variables presented included together in each model. *P* values <0.01 are in bold.

Singaporean adults,[29] and consistent with this, larger disc area has been found in participants who were more myopic in several studies.[51-53,63,65] While there is consistent evidence for myopia and longer axial length as risk factors for POAG,[66] height has not been reported as a risk factor for POAG. We did not find age to be independently associated with optic disc area; this is in agreement with the majority of the literature[16,17,21,44,64,67,68] and suggests that optic disc size does not alter significantly with time. We found women to have smaller discs, but this was explained largely by height (i.e., sex was not independently associated with disc size). Significantly smaller discs in women has been reported in the Baltimore Eye Survey[68] and the Tajimi Study,[30] but these were crude associations without adjustment for height. Several studies using confocal scanning laser ophthalmoscopy have found no statistically significant sex difference for disc size.[16,63,67,69-72]

Strengths of the present study include the population-based design and large sample size. Furthermore, detailed ophthalmic examination has been undertaken allowing adjustment for important confounders in analyses. There are limitations of the present study design. The EPIC-Norfolk Eye Study cohort is healthy and selected. It is likely that potential EPIC participants with significant visual impairment were not examined due to difficulty with required questionnaires or travel to the research clinic. Nevertheless, this would only result in a reduced study power to detect associations unless the direction of association was opposite in those not included, which is unlikely. Similarly, the exclusion of eyes without good quality HRT scans or complete data would most likely result in reduced power to detect associations, as it is unlikely that the associations examined would be in the opposite direction in the excluded eyes. The present study is cross-sectional, and therefore any causal inference is limited. The associations we found with age may be due to true longitudinal changes, or may be a cohort effect and we cannot be certain which of these is important from a cross-sectional study. A limitation of HRT is that many parameters rely on a reference plane, which is based on the position of a user-defined contour line. Therefore, there is a degree of subjectivity in the parameters given contour line

placement can vary even amongst experienced users.[73] Furthermore, physiological age-related decline in RNFL thickness will affect the position of the HRT reference plane, with a small posterior translation with increasing age. For any given optic nerve head, a more posterior reference plane is associated with a larger rim (and smaller cup) area estimate. Thus, the effect of a posterior translation of the reference plane with greater age is that the decline with age in *rim* (and increase in *cup*) observed in this study could be an underestimate. Another limitation of the HRT is that RNFL parameters are calculated relative to the reference plane at the disc margin, and may therefore be considered a surrogate measure rather than a true anatomical measure of the peripapillary RNFL. It should also be noted that while many of the associations we found were highly statistically significant, the coefficients were relatively small and this therefore limits any predictive ability of the regression models.

In summary, we found that HRT parameters in a population setting had three main components related to the cup, RNFL, and rim. We suggest examining at least one parameter related to each of these three components when assessing patients. The different components appear to have different associations within the present study cohort, potentially suggesting different contributory mechanisms for optic disc cupping and RNFL thinning.

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