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Relationship between retinal vessel diameter with both retinal nerve fibre layer thickness and optic nerve head parameters in middle-aged Caucasians: the Northern Finland Birth Cohort Eye study

Mohamed Geneid,[1,2,3,4] Joonas Kettunen,[1,2,3] Iida Nuuttila,[1,2,3] Timo Lintonen,[1,2,3] Janne-Joonas Uusitalo,[1,2,3] Ville Saarela[1,2,3] and M. Johanna Liinamaa[1,2,3]

[1]Department of Ophthalmology, Oulu University Hospital, Oulu, Finland
[2]MRC Oulu, University of Oulu, Oulu, Finland
[3]PEDEGO Research Unit, University of Oulu, Oulu, Finland
[4]Department of Ophthalmology, Päijät-Häme Central Hospital, Lahti, Finland

ABSTRACT.

Purpose: **To study the normal relationship between retinal vessel diameter (RVD) with retinal nerve fibre layer (RNFL) thickness and optic nerve head (ONH) parameters in a cohort of middle-aged Caucasians.**

Methods: **We investigated 3070 individuals (6140 eyes). Central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) were measured in the right eye using a semi-automated computer-assisted program. Retinal nerve fibre layer (RNFL) thickness and ONH parameters were assessed with Heidelberg retinal tomography (HRT).**

Results: **Data from 2217 persons were analysed including RNFL, CRAE, CRVE, sex, body mass index, mean arterial pressure, diabetes status, smoking status, optic disc area, rim area, spherical refraction and intraocular pressure. A larger RVD was associated with a thicker mean global RNFL thickness especially in global and inferior segments of the retina and with larger optic discs. Each 10 μm increase in the retinal arteriolar calibre was associated with a 5.58 μm increase in mean global RNFL thickness; the corresponding value for a 10 μm increase in venular calibre was 3.79 μm ($p < 0.001$ for both). Retinal venular calibre displayed consistent associations with RNFL thickness in both genders ($p < 0.001$ for all), whereas the association of arteriolar calibre and RNFL was more prominent in men ($p < 0.001$).**

Conclusion: **We found strong associations between larger RVD and thicker RNFL in all subjects. This study helps to clarify the association between RVD, RNFL thickness and ONH parameters and provides normal values for middle-aged Caucasians that will help in future studies investigating the role of vascular aetiology in systemic and eye diseases.**

Key words: central retinal arteriolar equivalent – central retinal venular equivalent – Heidelberg retinal tomography – IVAN – optic nerve head – retina

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Introduction

Retinal vessels are the only visible vessels in the human body, and by using noninvasive examination methods, associations have been reported between the retinal vessel diameters (RVD) and not only ocular but also systemic diseases, such as diabetes (Ikram et al. 2004), hypertension (Ikram et al. 2004, 2006; Liew et al. 2006), coronary heart disease (Wang et al. 2006; Wong et al. 2006; McGeechan et al. 2008) and atherosclerosis (Ikram et al. 2004; Smith et al. 2004; van Hecke et al. 2006). Several studies have detected correlations between the presence of glaucoma and RVD narrowing (Amerasinghe et al. 2008; Mitchell et al. 2005; Wang et al. 2007). Zheng et al. (2009) described a significant association between narrower retinal vessel calibre and retinal nerve fibre layer (RNFL) thinning in individuals with and without glaucoma. Furthermore, a correlation between RVD and RNFL has been observed by Jonas and Schiro (Jonas & Schiro 1993), who reported that a narrower RVD correlated with a thinner neuroretinal rim tissue. It has also been claimed that cerebral degenerative diseases cause changes in the thickness of the RNFL (Berisha et al. 2007; Gordon-Lipkin et al. 2007). According to those studies, there are associations between systemic

diseases, ocular diseases, RVD, RNFL thickness and optic nerve head (ONH) parameters.

The purpose of our study is to determine the association between RNFL thickness and ONH parameters with RVD in a large sample of middle-aged Caucasians. Understanding this association would not only determine normal values for middle-aged Caucasians but also help in future studies investigating systemic and eye diseases.

Materials and Methods

Study population

The original NFBC cohort consists of 12 058 subjects born in the region of Lapland and the Province of Oulu whose expected birth date was in 1966. This birth cohort has been followed prospectively since the gestational period. Between the years 2012 and 2014, the NFBC cohort were invited to attend an extensive clinical examination including physiological, cardiorespiratory, orthopaedic, dermatological, cognitive, dental status and ophthalmology examinations. Comprehensive laboratory tests were also taken. The study has received approval from the Ethical Committee of the Northern Ostrobothnia Hospital District. The current work was part of the NFBC Eye study whose design has been described in detail (Saarela et al. 2013). A total number of 3070 study subjects (6140 eyes) were screened. In 2779 subjects, data were available from both the Heidelberg retinal tomography (HRT) examination and fundus photos; in 2618 subjects, the right eye was analysed and in 161, it was the left eye. The left eye was used if the quality of fundus photos or HRT scans was not good enough in the right eye. Other variables were also assessed, that is gender, body mass index (BMI), mean arterial pressure, diabetes status, smoking status, optic disc area and intraocular pressure (IOP); all of this information was available for 2217 persons. The flow chart of the study population is shown in Fig. 1.

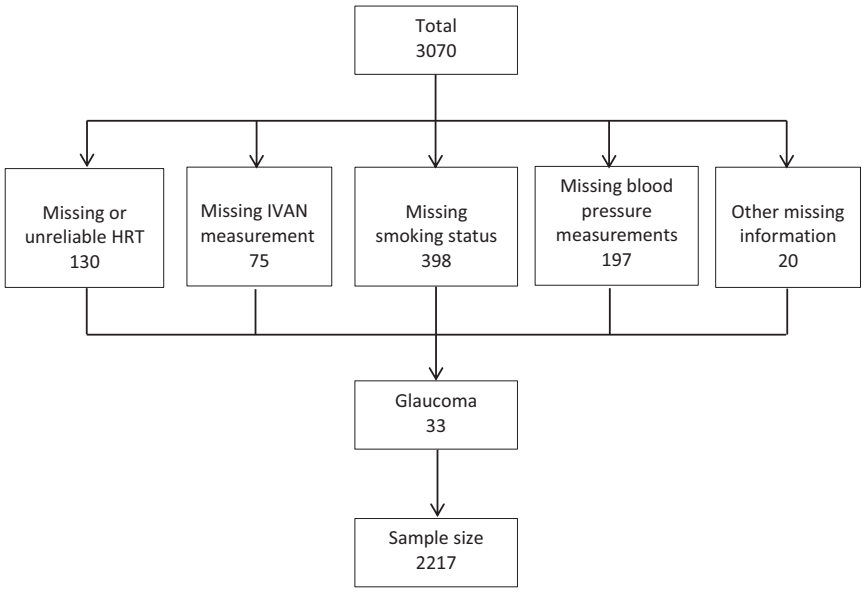

Fig. 1. Flow chart of the study population.

Retinal vessel diameters

Fundus photographs were obtained with a Canon CF-60DSi Digital Mydriatic Fundus Camera with attached Canon EOS-1Ds MK III SLR Digital Camera (Canon Inc., Tokyo, Japan). Retinal vessel diameter (RVD) indices were calculated using the Integrative Vessel Analysis (IVAN) software version 1.3 (Department of Ophthalmology and Visual Science, University of Wisconsin, Madison, WI, USA) from the fundus photographs. This software is a semi-automated system used to measure the width of retinal vessels from a digitized retinal image. The measurement of the RVD has been described in detail elsewhere (Wong et al. 2004). Briefly, six largest arterioles and venules passing completely through a circumferential zone 0.5–1 disc diameter from the optic disc margin were identified. The IVAN program automatically identified the optic disc and measured the diameters of these individual vessels. The grader ensured the correctness of vessel type selected by the program. Three summary variables were created by the software: the projected calibre size of the central retinal artery (CRAE), the projected calibre size of central retinal vein (CRVE) and the ratio of the two variables (arteriovenous ratio), using the revised Parr–Hubbard formula. In the current study, there were three graders, all masked with respect to the participants' characteristics and the intergrader variability was evaluated.

HRT imaging

The ONH topography was carried out after pupil dilation in a dim room using the Heidelberg Retina Tomograph (Heidelberg Engineering, Heidelberg, Germany; HRT3, image acquisition software version 3.1.2a, Heyex 1.6.2.0). A stack of captured images is used to form a three-dimensional topography image. The optic disc margin was manually defined by a trained photographer as the inner edge of Elschnig's ring. The HRT explores the ONH and the adjacent RNFL in stepwise progressing depths and provides indirect measurement of RNFL thickness, defined as the mean height of the 360° disc contour line from the reference plane. The stereometric ONH parameters (e.g. cup:disc area ratio and cup shape measure), Moorfield's regression analysis (MRA) and glaucoma probability score were calculated from the topography image. The stereometric ONH parameters (disc area, cup area, cup/disc area ratio, rim area, height variation contour, cup volume, rim volume, mean cup depth, max cup depth, cup shape measure, mean RNFL thickness, RNFL cross section area) as well as MRA statistics were calculated from the topography image. The HRT images were accepted if they had a topographic standard deviation <40 μm and a positive RNFL global index, indicating that the reference plane had been correctly positioned below the surface of the RNFL.

Cardiovascular, metabolic and ocular parameters

Systolic and diastolic blood pressure (BP) was measured to determine the

mean arterial pressure. Hypertension status was defined as all of the subjects who were using BP medication or whose minimum BP from three measurements was ≥140 mmHg for systolic or ≥90 mmHg for diastolic. Fasting blood samples were obtained to determine serum fasting glucose levels and diabetes status was defined as fasting plasma glucose ≥7.0 mmol/l or 2-hr plasma glucose ≥11.1 mmol/l, use of diabetic medication or a self-reported history of diabetes. Height and weight were measured to determine the BMI. A questionnaire was used to collect information about cigarette smoking. The measurement of refractive error was performed with the Nidek autorefractometer AR-360A and then subjectively by an optometrist and IOP was measured with rebound tonometry (iCare, Finland).

Table 1. Characteristics of the study persons.

	Included (n = 2217)	
	n	%
Men	911	41.1
Diabetes	122	5.5
Arterial hypertension	739	33.3
Current smoker	352	15.9
	Mean	SD
Plasma glucose, fasting (mM)	5.5	0.8
Systolic blood pressure (mmHg)	125	15.7
Diastolic blood pressure (mmHg)	85	10.6
Body mass index (kg/m^2)	26.8	4.9
Spherical refraction	−1.5	2.4
Intraocular pressure (mmHg)	14.8	3.4
Retinal arteriolar calibre (μm)	141.3	14.1
Retinal venular calibre (μm)	218.2	19.2
Mean retinal nerve fibre layer thickness (μm)	250.8	62.3
Disc area (mm^2)	2.2	0.5
Rim area (mm^2)	1.7	0.3
Rim volume (mm^3)	0.5	0.2

Statistical analysis

The statistical analyses were performed using IBM SPSS-statistics version 24 (IBM corporation, Chicago, IL, USA). Retinal vascular calibres (CRAE and CRVE) were analysed as continuous variables. Analysis of linear regression models was used to estimate the relationship of retinal vascular calibres with RNFL thicknesses and ONH parameters. Multiple linear regression models were used to estimate the difference in RNFL thickness for each standard deviation change in arteriolar and venular diameters, adjusted for gender.

Results

All subjects were born in the period 1965–1967 and their mean age at the time of data collection was 47.3 years (SD = 0.9). The characteristics of the examined subjects (n = 2217) are shown in Table 1. Some of the study subjects had to be excluded due to missing data (Fig. 1). Nonetheless, the baseline characteristics were similar between included and excluded persons except that the individuals who were included were slightly more myopic and had slightly lower BP than those excluded (data not shown) but these differences were not statistically significant.

There were three investigators who were grading the retinal vessel calibre data, and all three were masked with respect to the participant's characteristics in the current study. The intragrader variability was 4.5% for CRAE and 3.7% for CRVE; the intergrader variability was 3.7% for CRAE and 3.0% for CRVE.

We found that larger retinal arteriolar and venular calibres were associated with a thicker mean global RNFL thickness. Furthermore, CRAE and CRVE were associated with optic disc parameters, but CRVE was more significantly associated with optic disc area and rim area than with CRAE, while the rim volume was associated with both CRVE and CRAE. The data were adjusted for gender. The quintiles of CRAE and CRVE in relation to the global RNFL thickness and optic disc parameters in HRT are shown in Fig. 2.

A larger retinal arteriolar calibre was associated with an increased RNFL thickness markedly more often in men than women. Compared with retinal arterioles, retinal venular calibre displayed consistent associations with RNFL thickness in all subjects. Table 2 shows the associations of retinal vascular calibre with mean global RNFL thickness, stratified by gender.

After adjustment for gender, each 10 μm increase in retinal arteriolar calibre was associated with a 3.72 μm increase in mean global RNFL thickness ($p < 0.001$) and in the multivariate-adjusted analyses, each 10 μm increase in the retinal arteriolar calibre was associated with a 5.58 μm increase in mean global RNFL thickness ($p < 0.001$). The association was stronger in the inferior region of the retina. We also evaluated the relationship of retinal arteriolar calibre with ONH parameters in all subjects, after adjustment for gender. For every 10 μm increase in retinal arteriolar calibre, there was a 0.009 mm^3 increase in rim volume ($p < 0.001$) and a 0.006 mm increase in height variation contour ($p < 0.001$). In the multivariate-adjusted analyses, for every 10 μm increase in the retinal arteriolar calibre there was a 0.027 mm^2 increase in disc area ($p < 0.001$), a 0.023 mm^2 increase in rim area ($p < 0.001$), a 0.014 mm^3 increase in rim volume ($p < 0.001$) and a 0.007 mm increase in the height variation contour ($p < 0.001$). The relationship between retinal arteriolar calibre and RNFL thickness as well as with ONH parameters is demonstrated in Table 3.

Among all subjects, after adjustment for gender, each 10 μm increase in retinal venular calibre was associated with a 2.22 μm increase in mean global RNFL thickness ($p = 0.001$) and in the multivariate-adjusted analyses, each 10 μm increase in the retinal venular calibre was associated with a 3.79 μm increase in mean global RNFL thickness ($p < 0.001$) with the association being stronger in the inferior region of the retina. The relationships between retinal venular calibre with ONH parameters in

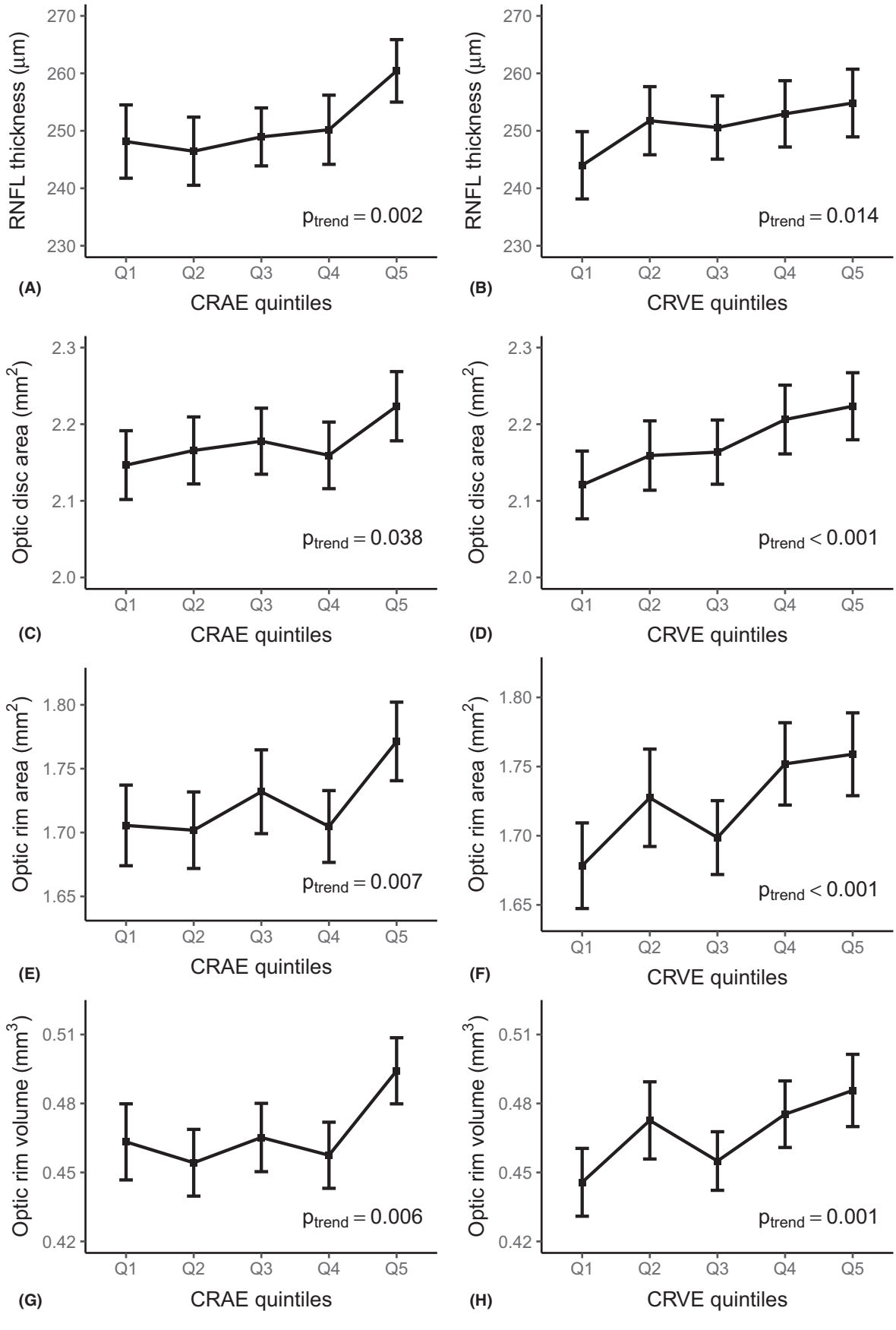

Fig. 2. Retinal vascular calibres were associated with retinal nerve fibre layer (RNFL) and optic disc parameters in Heidelberg retinal tomography. (A, B) The relationship of central retinal arteriolar equivalent (CRAE) and central retinal venular equivalent (CRVE) quintiles with mean global RNFL thickness. (C,D) The relationship of CRAE and CRVE quintiles with optic disc area. (E,F) The relationship of CRAE and CRVE quintiles with the optic rim area. (G,H) The relationship of CRAE and CRVE quintiles with the optic rim volume. The data have been adjusted for gender.

Table 2. Difference in retinal nerve fibre layer for each unit change in retinal vascular diameter.

				Multivariable-adjusted*	
	n	Mean (95% CI)	p	Mean (95% CI)	p
Retinal arteriolar calibre (μm)					
All persons	2217	0.32 (0.14 to 0.51)	0.001	0.56 (0.37–0.75)	<0.001
Men	911	0.72 (0.44 to 1.00)	<0.001	0.84 (0.55–1.13)	<0.001
Women	1306	0.17 (−0.07 to 0.40)	0.170	0.39 (0.14–0.64)	0.002
Retinal venular calibre (μm)					
All persons	2217	0.17 (0.03 to 0.30)	0.014	0.38 (0.25–0.51)	<0.001
Men	911	0.35 (0.15 to 0.56)	0.001	0.45 (0.25–0.66)	<0.001
Women	1306	0.14 (−0.03 to 0.32)	0.107	0.34 (0.16–0.51)	<0.001

* Adjusted for gender, body mass index, mean arterial pressure, diabetes status, current smoking status, optic disc area, spherical refraction and intraocular pressure.

Table 3. The association between retinal arteriolar calibre and retinal nerve fibre layer (RNFL) thickness as well as optic nerve head parameters.

	Gender-adjusted		Multivariable-adjusted	
	Mean (95% CI)	p	Mean (95% CI)	p
RNFL Thickness (μm) *				
Global	3.72 (1.91 to 5.53)	<0.001	5.58 (3.69 to 7.46)	<0.001
Temporal	0.18 (−0.51 to 0.88)	0.60	0.76 (0.02 to 1.50)	0.04
Temporal-to-superior	3.7 (1.28 to 6.11)	0.003	5.47 (2.88 to 8.06)	<0.001
Temporal-to-inferior	5.93 (3.50 to 8.37)	<0.001	6.97 (4.34 to 9.61)	<0.001
Nasal	2.82 (0.11 to 5.54)	0.04	5.16 (2.36 to 7.96)	<0.001
Nasal-to-superior	4.89 (2.09 to 7.70)	0.001	8.42 (5.46 to 11.39)	<0.001
Nasal-to-inferior	9.14 (6.39 to 11.89)	<0.001	10.37 (7.42 to 13.33)	<0.001
Optic disc parameters†				
Cup area (mm^2)	−0.001 (−0.011 to 0.01)	0.90	0.004 (−0.007 to 0.015)	0.50
Disc area (mm^2)	0.015 (0.001 to 0.029)	0.04	0.027 (0.012 to 0.042)	<0.001
Rim area (mm^2)	0.015 (0.006 to 0.025)	0.002	0.023 (0.013 to 0.034)	<0.001
Cup/disc area ratio	−0.001 (−0.005 to 0.002)	0.50	−0.001 (−0.004 to 0.003)	0.80
Cup volume (mm^3)	0.002 (−0.002 to 0.005)	0.30	0.004 (0.0002 to 0.008)	0.04
Rim volume (mm^3)	0.009 (0.004 to 0.014)	<0.001	0.014 (0.009 to 0.019)	<0.001
Mean cup depth (mm)	0.002 (−0.001 to 0.004)	0.20	0.003 (0.001 to 0.006)	0.01
Height variation contour (mm)	0.006 (0.003 to 0.008)	<0.001	0.007 (0.005 to 0.010)	<0.001

Data are the difference in RNFL thickness and optic disc parameters for every 10 μm increase in retinal arteriolar calibre with 95% CI values in parentheses.
* Adjusted for gender, optic disc area, body mass index, mean arterial pressure, diabetes and smoking status, spherical refraction and intraocular pressure.
† Adjusted for gender, body mass index, mean arterial pressure, diabetes and smoking status, spherical refraction and intraocular pressure.

all subjects were evident, that is each 10 μm increase retinal venular calibre was associated with a 0.014 mm^2 increase in rim area ($p < 0.001$), a 0.007 mm^3 increase in rim volume ($p < 0.001$) and a 0.004 mm increase in the height variation contour ($p < 0.001$). In the multivariate-adjusted analyses, each 10 μm increase in the retinal arteriolar calibre was associated with a 0.027 mm^2 increase in disc area ($p < 0.001$), a 0.023 mm^2 increase in rim area ($p < 0.001$), a 0.014 mm^3 increase in rim volume ($p < 0.001$) and a 0.007 mm increase in the height variation contour ($p < 0.001$). Table 4 illustrates the relationship between retinal venular calibre and the RNFL thickness as well as the ONH parameters.

Discussion

We studied the relationship between both RNFL thickness and ONH parameters with RVD in a population-based cohort of middle-aged Caucasians. Our study detected a significant association between RNFL thickness, ONH parameters and RVD, that is in those eyes with larger RVDs, we observed both thicker RNFL and larger optic discs. This study represents an excellent basis for revealing these physiological relationships in a homogeneous, well-documented, healthy adult population, since the imaging modalities have both high reliabilities and reproducibilities. In order to avoid the effect of confounding factors, the data were adjusted for gender, BMI, mean arterial pressure, diabetes status, smoking status, spherical refraction and IOP. These results can be used as a reference for the normal adult population in studies investigating diseases processes such as glaucoma, retinal and optic nerve diseases.

Previously, two relatively similar studies have been published, which examined RVD, ONH parameters and RNFL thickness. The first report was a population-based, cross-sectional study of Malay persons aged 40–80 years residing in Singapore (Zheng et al. 2009) conducted from 2004 to 2006. In concordance with our results, in that study, a reduced RNFL thickness was also associated with narrower vessels, with each SD decrease in retinal arteriolar calibre associated with a 5.81 μm decrease in the mean global HRT-measured RNFL thickness ($p < 0.001$) and each SD decrease in retinal venular calibre was associated with an 8.37 μm decrease in the mean global HRT-measured RNFL thickness ($p < 0.001$), although no such relationship was found in patients with glaucoma. The second study, which was a population-based study of healthy adolescents aged 12 years (Samarawickrama et al. 2009) examined in Australia, demonstrated that those eyes with thicker RNFL, thicker macula and larger optic discs exhibited larger retinal arteriolar and venular calibres, independent of age, sex, ethnicity, BMI, birth weight, axial length and mean arterial BP. In accordance with these studies, we now show that there are associations between RNFL thickness and ONH parameters that can be used as markers not only for ocular but also for systemic diseases in both the detection and follow-up of ocular pathologies affecting the RNFL.

In previous studies, associations have been found between the presence of systemic, eye diseases and RVD, for example between glaucoma and RVD narrowing (Mitchell et al. 2005; Wang et al. 2007; Amerasinghe et al. 2008;

Table 4. The association between retinal venular calibre and the retinal nerve fibre layer (RNFL) thickness as well as with optic nerve head parameters.

	Gender-adjusted		Multivariable-adjusted	
	Mean (95% CI)	p	Mean (95% CI)	p
RNFL thickness (μm)*				
Global	2.22 (0.88 to 3.56)	0.001	3.79 (2.46 to 5.11)	<0.001
Temporal	0.23 (−0.29 to 0.74)	0.40	0.66 (0.14 to 1.18)	0.01
Temporal-to-superior	2.63 (0.84 to 4.41)	0.004	4.02 (2.20 to 5.84)	<0.001
Temporal-to-inferior	3.95 (2.15 to 5.74)	<0.001	4.81 (2.96 to 6.67)	<0.001
Nasal	1.24 (−0.76 to 3.24)	0.20	3.56 (1.59 to 5.53)	<0.001
Nasal-to-superior	2.93 (0.86 to 5.00)	0.006	5.40 (3.32 to 7.49)	<0.001
Nasal-to-inferior	5.61 (3.58 to 7.64)	<0.001	6.57 (4.48 to 8.65)	<0.001
Optic disc parameters†				
Cup area (mm^2)	0.004 (−0.004 to 0.011)	0.30	0.003 (−0.005 to 0.011)	0.40
Disc area (mm^2)	0.017 (0.007 to 0.028)	0.001	0.024 (0.013 to 0.034)	<0.001
Rim area (mm^2)	0.014 (0.006 to 0.021)	<0.001	0.020 (0.013 to 0.028)	<0.001
Cup/disc area ratio	0.00 (−0.003 to 0.002)	0.90	−0.001 (−0.004 to 0.002)	0.50
Cup volume (mm^3)	0.003 (0.001 to 0.006)	0.01	0.003 (0.001 to 0.006)	0.01
Rim volume (mm^3)	0.007 (0.003 to 0.01)	<0.001	0.011 (0.008 to 0.014)	<0.001
Mean cup depth (mm)	0.001 (0.00 to 0.003)	0.10	0.002 (0.00 to 0.003)	0.06
Height variation contour (mm)	0.004 (0.002 to 0.006)	<0.001	0.006 (0.004 to 0.008)	<0.001

Data are the difference in RNFL thickness and optic disc parameters for every 10 μm increase in retinal venular calibre with 95% CI values in parentheses.

* Adjusted for gender, optic disc area, body mass index, mean arterial pressure, diabetes and smoking status, spherical refraction and intraocular pressure.

† Adjusted for gender, body mass index, mean arterial pressure, diabetes and smoking status, spherical refraction and intraocular pressure.

Tham et al. 2016). In other studies, also associations were detected between systemic diseases (such as coronary heart diseases) and RVD narrowing (Wang et al. 2006; Wong et al. 2006; McGeechan et al. 2008). Another important finding emerged from a population-based cohort study of 3654 Australians aged at least 49 years; it was found that a smaller retinal arteriole to venule ratio and narrower retinal arterioles were associated with coronary heart disease death (Wang et al. 2006). Furthermore, when retinal abnormalities were examined in patients with early Alzheimer's disease, a significant thinning of the RNFL was found in the superior quadrant (Berisha et al. 2007) and a similar association was detected when RNFL thickness was evaluated in conjunction with multiple sclerosis, that is, RNFL thickness was associated with brain parenchymal fraction and cerebrospinal fluid volume (Gordon-Lipkin et al. 2007). Therefore, as it is important to assess objectively the effect of systemic or eye diseases on RVD, it is essential that such normal values and anatomical correlations as described in this study are available to form a basis for future studies.

Several factors influence retinal vascular calibre; in previous studies, it has been observed that arteriolar calibre is influenced by age, gender, race, BP and incident hypertension, obesity, lipids and cigarette smoking (Samarawickrama et al. 2009; Sun et al. 2009; Zheng et al. 2009). Furthermore, retinal venular calibre has been found to be influenced by gender, race, BP and incident hypertension, obesity, lipids and cigarette smoking (Sun et al. 2009). Since our study was aimed at obtaining normative values, we wanted to adjust for confounding factors. Therefore, the data we show here are adjusted for gender, mean arterial pressure, BMI, diabetes status, IOP, refraction, smoking status and disc area. The study subjects were of a similar age and all of them were Caucasian; therefore, no adjustment was needed for age or race. Since the changes in data are relatively small, we believe that these kinds of adjustment are necessary for ensuring the reliability of the results. These kinds of data adjustment have also been used in previous studies (Samarawickrama et al. 2009; Zheng et al. 2009).

The RNFL thickness in our study was correlated with RVD; that is, the thickest RNFL was found in the highest quintiles of CRAE or CRVE. This has also been observed before by several investigators (Samarawickrama et al. 2009; Zheng et al. 2009). Although it is impossible to provide definite answers to this question, it is possible that during fetal development, wider vessels give better nutritional support to the developing RNFL and therefore secondarily influence the RNFL thickness. Furthermore, it is plausible that these changes are related to vascular autoregulation, since physiological levels of factors such as nitric oxide have been found to evoke vasodilatation and to elevate blood flow (Toda & Nakanishi-Toda 2007) and therefore to influence RVD. In contrast, nitric oxide activity decreases in glaucoma (Nathanson & McKee 1995). Furthermore, the narrowing of RVD is crucial in certain diseases; for instance, it may be related to the development of normotensive glaucoma, since RVDs have been observed to be narrower in patients with normotensive glaucoma (Chang et al. 2011). Further prospective studies will be needed in order to assess if retinal vascular narrowing at an earlier age is a risk factor for (normotensive) glaucoma later in life.

Furthermore, the smallest optic disc area was found in lowest quintiles of CRAE and CRVE. In addition, the smallest rim volumes and areas were observed in the lowest quintiles of CRAE and CRVE. However, we did not find any correlation between RVD and cup area, cup volume or cup:disc area ratio. Previously, such an association between RVD and cup area, cup volume or cup:disc area has also been lacking (Samarawickrama et al. 2009). It has been shown that the risk for nonarteritic anterior ischaemic optic neuropathy (NAION) was elevated in subjects with a small optic cup-to-disc ratio (Burde 1993), and therefore, possibly a smaller disc and a narrower RVD may describe mechanistic pathways involved in the development of NAION. However, since there were no NAION cases nor was the association for cup measurements detected in the current study, it is impossible to draw definite conclusions about the plausibility of this link. However, a longitudinal study investigating this topic is a part of the current project and it will hopefully provide definitive results.

The strengths of our study are its large population-based sample size, and the fact that NFBC provides a homogenous

population in terms of age, ethnicity and living environment. This is a population-based birth cohort which is of Caucasian origin and which was now examined when they were 46–48 years old. Another strength of our study is the standardized assessment of the diameters of the retinal vessels, RNFL and disc parameters of healthy eyes. Potential eye diseases, such as glaucoma, were well documented and these cases were excluded. Furthermore, this cohort has been well documented and standardized measurements were gathered for refraction, other eye variables, biometry and anthropometric measurements (Saarela et al. 2013). However, one limitation of the study is that rather few subjects were suffering from conditions such as diabetes or hypertension which might influence the retinal circulation. Nonetheless, these diseases were well documented in the current study, diabetes and hypertension were considered as confounding factors, and the data were adjusted for these conditions. Another limitation is that the cross-sectional design of the study may limit conclusions about causality. Furthermore, we cannot rule out certain random errors, such as the timing of photography in relation to the cardiac cycle, although the large sample size should diminish this possibility.

In summary, we detected significant inter-relationships between RNFL thickness, ONH parameters and RVD. These represent anatomical normal values of RNFL thickness, ONH parameters and RVD for middle-aged Caucasians and they will help in future studies investigating the vascular aetiology in eye diseases affecting also RNFL or ONH and also assist in differentiating between pathological and physiological findings.

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Correspondence:
M. Johanna Liinamaa
Department of Ophthalmology
Oulu University Hospital
Box 21, 90029
Oulu, Finland
Tel: (+358) 8-315 3727
Fax: +358 831 533 39
Email: johanna.liinamaa@oulu.fi

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