U-Shaped Effect of Blood Pressure on Structural OCT Metrics and Retinal Perfusion in Ophthalmologically Healthy Subjects

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PURPOSE. We wanted to investigate the association of blood pressure (BP) status with the ganglion cell-inner plexiform layer (GCIPL) and retinal nerve fiber layer (RNFL) thickness of nonglaucomatous eyes and to elucidate whether this effect is related to vascular metrics proxying retinal perfusion.

METHODS. For this case-control study, we prospectively included 96 eyes of 96 healthy subjects (age 50–65) from a large-scale population-based cohort in the northern Netherlands (n = 167,000) and allocated them to four groups (low BP, normal BP [controls], treated arterial hypertension [AHT], untreated AHT). We measured macular GCIPL and RNFL (mRNFL) and peripapillary RNFL (pRNFL) thicknesses with optical coherence tomography (OCT). We estimated retinal blood flow (RBF), retinal vascular resistance (RVR), and autoregulatory reserve (AR) from quantitative OCT-angiography, fundus imaging, BP, and intraocular pressure. We compared structural and vascular metrics across groups and performed mediation analysis.

RESULTS. Compared to controls, GCIPL was thinner in the low BP group ($P = 0.013$), treated hypertensives ($P = 0.007$), and untreated hypertensives ($P = 0.007$). Treated hypertensives exhibited the thinnest mRNFL ($P = 0.001$), temporal pRNFL ($P = 0.045$), and inferior pRNFL ($P = 0.034$). The association of GCIPL thickness with BP was mediated by RBF within the combined low BP group and controls ($P = 0.003$), by RVR and AR within the combined treated hypertensives and controls ($P = 0.001$, $P = 0.032$), and by RVR within the combined untreated antihypertensives and controls ($P = 0.022$).

CONCLUSIONS. Inner retinal thinning was associated with both tails of the BP distribution and with ineffective autoregulation. Longitudinal studies could clarify whether these defects can explain the reported glaucomatous predisposition of these population groups.

Keywords: optical coherence tomography, blood pressure, retinal blood flow, autoregulation, glaucoma

Glaucoma is a chronic optic neuropathy characterized by thinning of the retinal nerve fiber layer (RNFL), loss of retinal ganglion cells (RGCs), and progressive visual function decline.[1] Although elevated intraocular pressure (IOP) is considered the most important modifiable risk factor, glaucoma may also manifest in those with apparently normal IOP (normal-tension glaucoma [NTG]).[2–4] Therefore certain components of the disease remain elusive or insufficiently addressed.

It has been proposed that low or unstable blood supply could lead to reduced oxygenation of the RGCs.[4,5] From a hemodynamic perspective, blood flow is determined by the balance between ocular perfusion pressure (OPP) and vascular resistance.[6] Therefore low blood pressure (BP) could result in low OPP, thus increasing the risk for glaucoma incidence and progression, possibly because of flow-mediated damage to the RGCs. Indeed, this has been observed in some cross-sectional and longitudinal population-based studies.[7–10] However, other studies do not confirm this finding, and there is also evidence that this association becomes relevant only when low BP manifests as pronounced nocturnal dipping.[11,12] On the other hand, although arterial hypertension (AHT) is also frequently reported as a risk factor for glaucoma, conflicting results exist on whether BP reduction exacerbates or protects from glaucomatous optic neuropathy (GON), possibly depending on individual medication effects and on how aggressive the treatment strategy is.[13–20]

Current assessment of RGC structure is based on optical coherence tomography (OCT), whereas its more recent extensions, OCT-angiography (OCT-A) and Doppler OCT, enable the noninvasive evaluation of retinal perfusion.[21–23] These imaging modalities have already revealed that, in glaucoma patients, reduced blood flow is associated with

visual field (VF) deterioration, independently of neural tissue damage.[24–26] However, perfusion deficits could, at the same time, be the cause (low supply) or consequence (low demand) of GON. This realization is known as the "chicken-egg" dilemma in glaucoma. Therefore, without first establishing a clear picture of the baseline interplay between vascular risk factors (in this case BP status), perfusion, and structure in healthy eyes, it is difficult to objectively assess GON for a potential vascular component.

The retinal microcirculation normally demonstrates the ability of autoregulation, that is, active modification of vascular caliber in response to local signals, keeping retinal blood flow (RBF) essentially constant.[27] This property protects the tissue from ischemia, in case of OPP drops. BP status can interfere with this process: BP lowering brings subjects closer to their lower autoregulation limit (LARL), thus at risk of hypoperfusion, whereas AHT may cause endothelial damage and flow dysregulation.[28] In this study, we hypothesized an inverse U-shaped association between BP status and structural OCT measures in nonglaucomatous eyes. Although the detrimental effect of AHT to the RGCs and their axons has been previously documented, this effect has not been studied in subjects with low BP, nor has it been examined in combination with RBF autoregulation.[29–32] Studies until now have used linear models to describe the association between BP and structural OCT measures, thus potentially neglecting any signal coming from the left tail of the distribution.[33,34]

Therefore the aims of this study were to investigate the effect of low BP, treated AHT, and untreated AHT on the inner retinal layer thicknesses of non-glaucomatous eyes and to elucidate whether this effect is related to retinal perfusion and to crossing the lower limit of RBF autoregulation. For this purpose, we performed multimodal structural and vascular imaging in ophthalmically healthy normotensive controls, treated arterial hypertensives, and individuals belonging to the lower and higher (untreated) tails of the BP distribution. Participants were selected from the large-scale population-based Lifelines cohort, which enabled us to study the real extremes, especially from the low BP tail, in an unbiased manner.

METHODS

Study Design and Population

For this cross-sectional, case-control study, we prospectively recruited subjects via targeted invitation among the participants of a large-scale prospective cohort study of the northern Netherlands (Lifelines Biobank; n = 167,000).[35] Subjects were invited solely on the basis of their BP status and age. After a strict selection procedure, 105 participants between 50 and 65 years of age satisfied both the BP criteria (see next paragraph) and the ophthalmic and medical history inclusion criteria: unoperated eyes; best-corrected visual acuity $\geq$ 0.8; spherical refractive error between -3 and $+3$ D; cylinder not exceeding 2 D; IOP $\leq$ 21 mm Hg (non-contact tonometer Tonoref II; Nidek, Aichi, Japan); no reproducibly abnormal VF test locations (Frequency Doubling Technology [C20-1 screening mode]; Carl Zeiss, Jena, Germany); no family history of glaucoma; no ophthalmic, hematologic, or cardiovascular disease (except for AHT), and no diabetes. We performed additional documentation of ophthalmic health with the subsequent imaging sessions (see *Data collection*).

We allocated participants to one of four non-overlapping groups: (1) low BP, (2) normal BP (controls), (3) treated AHT, and (4) untreated AHT (see next paragraph). All participants provided written informed consent. The ethics board of the University Medical Center Groningen approved the study protocol (no. NL61508.042.17). The study followed the tenets of the Declaration of Helsinki.

Blood Pressure Group Definitions

We defined low BP (group 1) as both systolic and diastolic BP (SBP, DBP) lower than the 10th percentiles of the Lifelines Biobank age-matched population (110 mm Hg and 65 mm Hg, respectively), without any AHT record. This criterion had to be confirmed on at least two previous, separate occasions (ascertaining that subjects truly belonged to the tail of the distribution and did not regress towards the mean). We defined untreated AHT (group 4) similarly, with the criteria being both SBP and DBP higher than the 90th percentiles of the Lifelines Biobank age-matched population (149 mm Hg and 88 mm Hg, respectively), verified at least twice previously. Subjects of this group were aware of their BP status, but never made use of antihypertensive medication, by choice. For the aforementioned groups, recruitment started from the tails of the SBP and DBP distributions (outside the first and ninety-ninth percentile), moving upward toward the tenth percentile bound for group 1 and downward toward the ninetieth percentile bound for group 4. For treated AHT (group 3), we randomly invited participants documented as receiving (and still making uninterrupted use of) antihypertensive medication for at least one year. Last, we defined normal BP (group 2) as both SBP and DBP within 1 standard deviation (SD) from the mean of the age-matched population (SBP: 113 mm Hg to 143 mm Hg and DBP: 67 mm Hg to 85 mm Hg, measured on site) and no previous record of AHT. For all groups, recruitment ended upon group completion or upon unavailability or unresponsiveness of participants with the required characteristics. Specifically, the power analysis for a one-way ANOVA design (power, 0.8; alpha level, 0.05; effect size f, 0.35; groups, 4) recommended 24 subjects per group, which was rounded up to 25.

The definitions used for group 1 (low BP) and group 4 (untreated AHT) are based on predetermined cutoffs of an otherwise continuous variable (BP), based on multiple previous measurements. To verify the robustness of statistical findings and allow for direct comparison with relevant studies, for these groups we also used the standard, cross-sectional definition, based on the on-site BP measurement. Specifically, low/high SBP was defined as SBP outside the 5th/95th percentile of the Lifelines Biobank population (105 mm Hg and 155 mm Hg, respectively). The same definition was used for low/high DBP (61 mm Hg and 92 mm Hg, respectively).

Data Collection

All participants were examined at the same time of the day (5:00 PM–6:30 PM) and were not given any instructions regarding their routine before their visit. After screening (see previous section), we applied mydriatic drops that have been shown not to affect RBF (tropicamide 0.5%).[36] After the participants had rested in a quiet room for 20 minutes, we recorded BP from the brachial artery, in sitting position, with
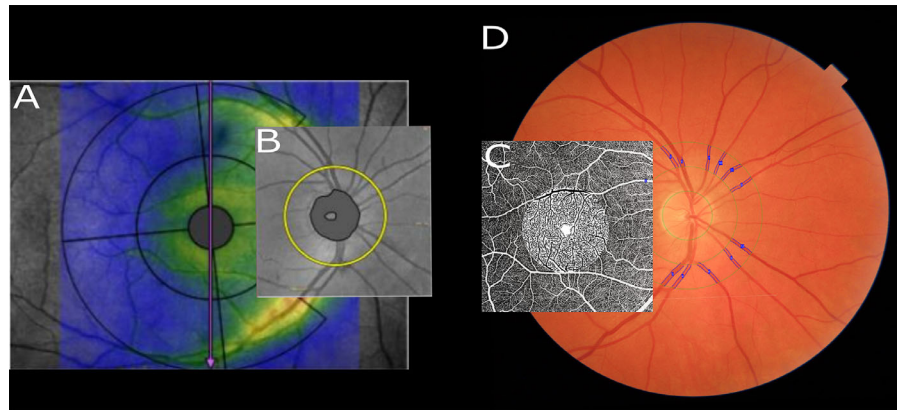
FIGURE 1. Structural (**A, B**) and vascular (**C, D**) regions of interest. (**A**) The mRNFL and GCIPL thicknesses measured within the larger (10 mm diameter) circle centered at the fovea, excluding the innermost 1 mm diameter circle and the nasal sector of the outer ring. (**B**) The pRNFL measured at a circle of 3.45 mm diameter centered at the ONH. (**C**) OCT-A scan of 6×6 mm centered at the fovea. Signal intensity inside the innermost 3-mm diameter circle is binarized in flow (*black*) and non-flow (*white*). (**D**) A 45° fundus image centered at the ONH. The six largest arterioles and six largest venules between rings of two and three optic disc diameters are marked in *blue*.

an automatic monitor (Omron M6 Comfort, Omron Healthcare, Kyoto, Japan). We averaged two readings, unless there was a discrepancy of at least 10 mm Hg in SBP or 5 mm Hg in DBP, in which case we averaged three readings. We also measured the weight and height of each participant.

For the imaging session, we selected, randomly if both eyes fulfilled the inclusion criteria, one eye per participant. We performed macular and optic nerve head (ONH) structural OCT imaging, as well as parafoveal OCT-A (Canon HS100 SD-OCT; Canon, Inc., Tokyo, Japan). The device automatically segments and quantifies macular RNFL (mRNFL) thickness and ganglion cell–inner plexiform layer (GCIPL) thickness within a 10-mm diameter circular region of interest (ROI) centered at the fovea (Fig. 1A), a region that has been shown to be advantageous over the commonly used 5-mm diameter ROI for mRNFL measurements.[37] It also reports peripapillary RNFL (pRNFL) thickness at a 3.45-mm diameter circle centered at the ONH (Fig. 1B). We further subdivided pRNFL into temporal, superior, nasal, and inferior and also recorded values for the neuroretinal rim (NRR) area and the vertical cup-to-disc ratio (VCDR). We additionally acquired two 6×6 mm OCT-A scans centered at the fovea (Fig. 1C). We required an image quality of 7/10 or better, as well as the absence of any artifacts or segmentation errors for all OCT and OCT-A scans, resulting in the exclusion of 9 out of the 105 subjects.

After registering and binarizing the signal of the en face OCT-A images, we calculated the fractal dimension (FD) of the superficial vascular plexus, inside a 3-mm diameter circle centered at the fovea (Fig. 1C). We have previously provided details on FD and its calculation, as well as on the specifications and repeatability of the Canon OCT-A.[38,39] In short, FD represents the complexity of the branching pattern and is lower in conditions with sparser vasculature, such as glaucoma.[40]

Lastly, we acquired two 45° high-quality and artifact-free fundus images (TRC-NW400; Topcon Corporation, Tokyo, Japan), centered at the ONH (Fig. 1D). For Gullstrand's schematic eye, that is, not accounting for variations in corneal curvature or axial length and assuming a distance of 17 mm between the secondary nodal point and the retina, the resolution of this camera is ~6.9 μm per pixel. For each image, we derived the central retinal artery and vein

equivalents (CRAE, CRVE; i.e., diameters) using the standardized Knudtson-Parr-Hubbard iteration, whose details and validation can be found elsewhere.[38,41] In short, we back-calculated vessel diameters using the six largest arteriolar and six largest venular branches, identified within a ring centered at the ONH (2 and 3 optic disc diameters). We recorded the average CRAE and CRVE of two images.

Retinal Blood Flow and Lower Autoregulation Limit

We calculated total retinal vascular resistance (RVR) using the measured FD, CRAE, and CRVE of each participant, as well as population-based hematocrit values (Lifelines Biobank), adjusted for age, sex, and blood pressure status. We have previously documented the mathematics behind this Poiseuille-based model and its validation in vivo, by means of Laser Speckle Flowgraphy (LSFG).[38,42] Subsequently, we computed total RBF, using RVR and retinal perfusion pressure (RPP), a more precise estimation of OPP for the retinal circulation[38]

$$RBF = \frac{RPP}{RVR} \quad (1)$$

where RPP $= (0.39 \cdot MAP + 10.1) - IOP$ mmHg and MAP $= \frac{1}{3}SBP + \frac{2}{3}DBP$ is the mean arterial pressure.

We defined LARL as the lowest RPP value for which RBF can be maintained constant (Fig. 2). At this critical point, the vasculature has reached its maximal autoregulatory capacity and any further pressure drop will not trigger compensatory vasodilation, resulting in flow reduction. We have previously shown and experimentally validated, by means of LSFG, that LARL can be approximated as

$$LARL = RBF \cdot RVR_{max} \quad (2)$$

where RVR_{max} is an upper bound observed in a population.[38]

In this study, we defined RVR_{max} as the 95th percentile of the RVR distribution. Due to the possible occurrence of structural remodeling in retinal vessels belonging to subjects with AHT, we separated the RVR distributions of the
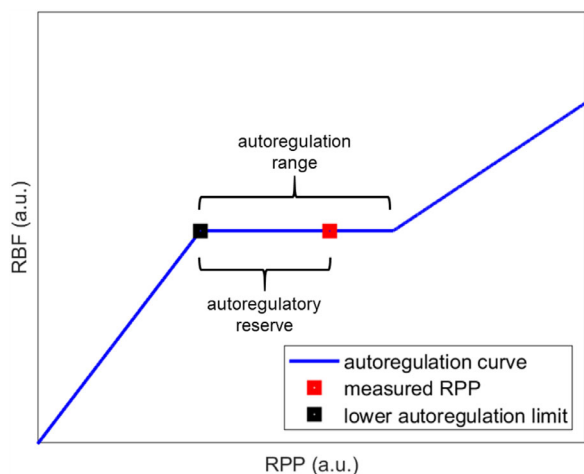
FIGURE 2. Theoretical autoregulation curve (axes in arbitrary units). RBF is displayed as a function of RPP. Within the autoregulation range, blood flow is maintained constant. The distance of the actual (measured) RPP from the LARL is the AR.

non-hypertensives (groups 1 and 2) and hypertensives (groups 3 and 4).[6]

Last, for each participant, we defined the *autoregulatory reserve* (AR) as the difference between measured RPP and predicted LARL (Fig. 2).

Statistical Analysis

This study is divided in three parts. For the first part of the analysis (structural OCT analysis), to establish the existence of a U-shaped association (if any), we univariably compared structural OCT metrics (mRNFL, GCIPL, and pRNFL) between the four BP groups, without accounting at this point for any vascular factors. For the second part of the analysis, to investigate whether any vascular factors could possibly explain this association, we univariably compared RVR, RBF, and AR between the groups. For the last part of the analysis, we performed mediation analysis to examine whether the vascular metrics lie in the explanatory pathway of the relationship between MAP and the structural OCT metrics that were significant in the first part of the analysis. All analyses were initially performed using the recruitment BP definitions and were reiterated using the standard, cross-sectional definitions based on the fifth and ninety-fifth percentiles of the SBP and DBP distributions (see *Blood pressure group definitions*).

We described normally distributed variables with the mean and standard deviation (SD) and variables with a skewed distribution with the median and interquartile range (IQR). We used one-way ANOVA with post hoc tests for group mean comparisons, adjusting for potential confounders. To account for multiple testing, we implemented the Tukey HSD correction. We applied Levene's test to check for equality of variances. Whenever ANOVA assumptions were not met, we used nonparametric tests, Welch's one-way ANOVA with the Games-Howell correction, or quantile regression.

To determine a mediation effect, we used Baron and Kenny's mediation steps.[43] In short, a vascular factor M was considered a mediator of the effect of MAP (X) on struc-

tural OCT (Y), if the following were true in linear regression analysis:

a. X was a significant predictor of Y (Y~X).
b. X was a significant predictor of M (M~X).
c. When M was added to the model (Y~X+M), M was a significant predictor of Y and the significance of X as a predictor of Y was reduced.

We verified these findings by using the Sobel test for indirect effects. Mediation analysis was performed separately for the low BP group together with the controls, the treated AHT group together with the controls, and the untreated AHT group together with the controls. RBF, RVR, and AR were examined as potential mediators.

All analyses were performed using R (version 3.3.3; R Foundation for Statistical Computing, Vienna, Austria) and SPSS (version 26; IBM Corp., Armonk, NY). $P \leq 0.05$ was considered statistically significant.

RESULTS

In total, 96 eyes of 96 subjects fulfilled all the criteria and were included in the analysis. Table 1 displays the characteristics of the population, stratified by BP status. Sex and body mass index (BMI) were significantly different between groups, aside from BP. As expected, the low BP group comprised almost exclusively females, whereas higher BMI was present in the hypertensive groups.[44,45] Other factors that could affect the comparisons, such as age, IOP, spherical equivalent (SEQ), and ONH area, were similar between groups.

Structural Metrics

Table 2 and Figure 3 present the comparison of structural OCT metrics across the four BP groups (recruitment BP definitions). Adjusted post hoc comparisons revealed that, compared to the controls, GCIPL was significantly thinner in the low BP group ($P_{adj} = 0.013$), the treated AHT group ($P_{adj} = 0.007$), and the untreated AHT group ($P_{adj} = 0.007$). The mRNFL was also thinner, but this was only significant for the treated AHT group ($P_{adj} = 0.001$). Interestingly, mRNFL in treated hypertensives was even significantly thinner than in untreated hypertensives ($P_{adj} = 0.033$). Figure 3 shows the characteristic (inverse) U shape for the macular OCT metrics. There was no clear effect of BP group on the mean pRNFL, the NRR area, or the VCDR. However, treated hypertensives had a thinner temporal pRNFL ($P_{adj} = 0.045$) than normotensives. Also, inferior pRNFL was borderline thinner in subjects with low BP ($P_{adj} = 0.083$) and clearly thinner in both treated and untreated hypertensives ($P_{adj} = 0.034$ and 0.033, respectively). Sex, SEQ, and BMI did not confound any of the associations (all *P* values > 0.05), and this was still true after the omission of any group from the analysis.

The structural comparisons using the 5th/95th percentile definition cutoffs are presented in Supplementary Tables S1 (SBP) and S2 (DBP). Associations were consistent and adjusted significances remained essentially unchanged.

Vascular Metrics

Figure 4 displays the vascular outcome variables (RBF, RVR, and AR) as a function of BP status (recruitment BP definitions). For reference, Table 3 presents RPP, hematocrit, and

TABLE 1.   Characteristics of the Study Population

	Group 1 (Low BP)	Group 2 (Normal BP)	Group 3 (Treated AHT)	Group 4 (Untreated AHT)	P Value
Group size *(N)*	31	21	26	18	
Age (y), mean (SD)	56.1 (4.4)	55.9 (4.7)	56.4 (4.8)	57.2 (4.6)	0.81
Sex, % female	93.5	47.6	42.3	44.4	**<0.001**
SBP (mm Hg), mean (SD)	106 (9)	126 (6)	142 (18)	159 (22)	**<0.001**
DBP (mm Hg), mean (SD)	66 (6)	79 (6)	86 (11)	99 (8)	**<0.001**
BMI (kg · m^{-2}), median (IQR)	22.1 (21.2 to 24.3)	23.3 (22.1 to 26.5)	26.9 (24.7 to 29.8)	27.3 (24.3 to 28.4)	**<0.001**
Smoking, % exposed	22.6	38.1	30.8	38.9	0.57
IOP (mm Hg), mean (SD)	13.9 (3.0)	13.2 (3.1)	14.3 (3.0)	14.6 (3.7)	0.56
SEQ (D), mean (SD)	−0.10 (1.41)	0.27 (1.67)	−0.23 (1.55)	−0.68 (1.69)	0.31
ONH area (mm^2), median (IQR)	1.89 (1.69 to 2.24)	1.96 (1.71 to 2.20)	1.94 (1.72 to 2.31)	2.00 (1.78 to 2.29)	0.75

TABLE 2.   Structural OCT Metrics as a Function of BP Status

	Group 1 (Low BP)	Group 2 (Normal BP)	Group 3 (Treated AHT)	Group 4 (Untreated AHT)	P Value
GCIPL (µm), mean (SD)	53.6 (2.7)	56.2 (3.0)	53.4 (3.3)	53.1 (2.5)	**0.002**
mRNFL (µm), mean (SD)	37.7 (3.3)	39.8 (3.4)	36.0 (2.8)	38.7 (3.4)	**0.001**
Total pRNFL (µm), mean (SD)	99.4 (8.2)	103.1 (10.1)	96.9 (9.2)	100.3 (8.8)	0.14
Temporal pRNFL (µm), mean (SD)	71.8 (10.5)	74.0 (8.1)	65.5 (9.5)	72.6 (15.3)	**0.038**
Superior pRNFL (µm), mean (SD)	119.6 (14.7)	124.1 (16.7)	119.5 (13.5)	126.1 (16.6)	0.36
Nasal pRNFL (µm), mean (SD)	82.2 (11.2)	80.6 (14.1)	81.4 (11.4)	80.6 (14.0)	0.96
Inferior pRNFL (µm), mean (SD)	124.5 (12.9)	134.0 (13.3)	122.7 (14.3)	121.5 (15.7)	**0.019**
NRR area (mm^2), median (IQR)	1.43 (1.21 to 1.63)	1.37 (1.27 to 1.74)	1.35 (1.18 to 1.64)	1.52 (1.41 to 1.69)	0.50
VCDR, median (IQR)	0.54 (0.42 to 0.60)	0.49 (0.39 to 0.55)	0.47 (0.42 to 0.60)	0.51 (0.39 to 0.59)	0.82

the components measured by fundus imaging (CRAE, CRVE) and OCT-A (FD) that were used in the calculation of the vascular outcome variables. There were differences in RBF between groups ($P = 0.034$), but after adjusting for multiple comparisons RBF was only significantly lower in the low BP group when compared to the untreated AHT group ($P_{adj} = 0.043$). RVR was also different between groups ($P = 9.0 \cdot 10^{-9}$), with the additional presence of a larger variance in the treated AHT group (Levene's test: $P = 0.002$). With regards to AR, the unequal variances were also statistically significant (Levene's test: $P = 0.0002$), showing that, unlike any other group, treated hypertensives could have either a large or a small AR. As can be better seen in Figure 5 and Supplementary Table S3, the low BP group had a significantly smaller AR than the control group, regardless of AR quantile compared. Conversely, the untreated hypertensives had a significantly larger AR than the control group, regardless of quantile compared. However, there was a mixed response in the treated AHT group: the AR was significantly smaller than that of the controls for small quantiles, although it was similar or larger for larger quantiles. In addition, correlation analysis within the treated AHT group revealed that the smaller AR quantiles corresponded to the lowest MAP values (Pearson's $r = 0.45$, $P = 0.020$), that is, to the most intensively controlled hypertensives. Again, sex, SEQ, and BMI did not confound these associations. The use of the fifth/ninety-fifth percentile–based BP definitions resulted, again, in almost identical findings (data not shown).

Mediation Analysis

Results from mediation analysis regarding the effect of BP status on GCIPL are presented in Table 4 (recruitment BP definitions). RBF was mediating the association of GCIPL with BP within the combined low BP group and controls, while RVR was mediating the same association within the combined untreated AHT group and controls. RVR and AR were both independently mediating the association of GCIPL with BP within the combined treated AHT group and controls. In the complete model (GCIPL~MAP + RVR + AR), which accounts for the covariance between RVR and AR, the opposite, real effect of AR became visible, that is, small AR was associated with thinner GCIPL (see Discussion section). We did not observe any vascular mediation for the effect of BP status on RNFL metrics.

Supplementary Tables S4 and S5 present the results of the same mediation analysis, using the fifth/ninety-fifth percentile-based BP definitions. Interestingly, similar associations, but with slightly different patterns were present in the GCIPL-SBP analysis (S4) versus the GCIPL-DBP analysis (S5). Although low RBF can account for the association of low SBP with GCIPL thinning, it does not account as much for the association of low DBP with GCIPL thinning. In addition, in the AHT groups, DBP is a more important determinant of RVR and AR, compared to SBP.

DISCUSSION

In this study, we reported three main findings. In the first part of the analysis (structural OCT analysis) we uncovered an inverse U-shaped association between blood pressure status and OCT metrics (GCIPL and RNFL), with both low and high blood pressure being associated with thinning of the inner retinal layers. In the second part of the analysis (analysis of vascular metrics), we showed that, despite the existence of retinal blood flow autoregulation, only a small autoregulatory reserve is present in individuals with low blood pressure, as well as in individuals
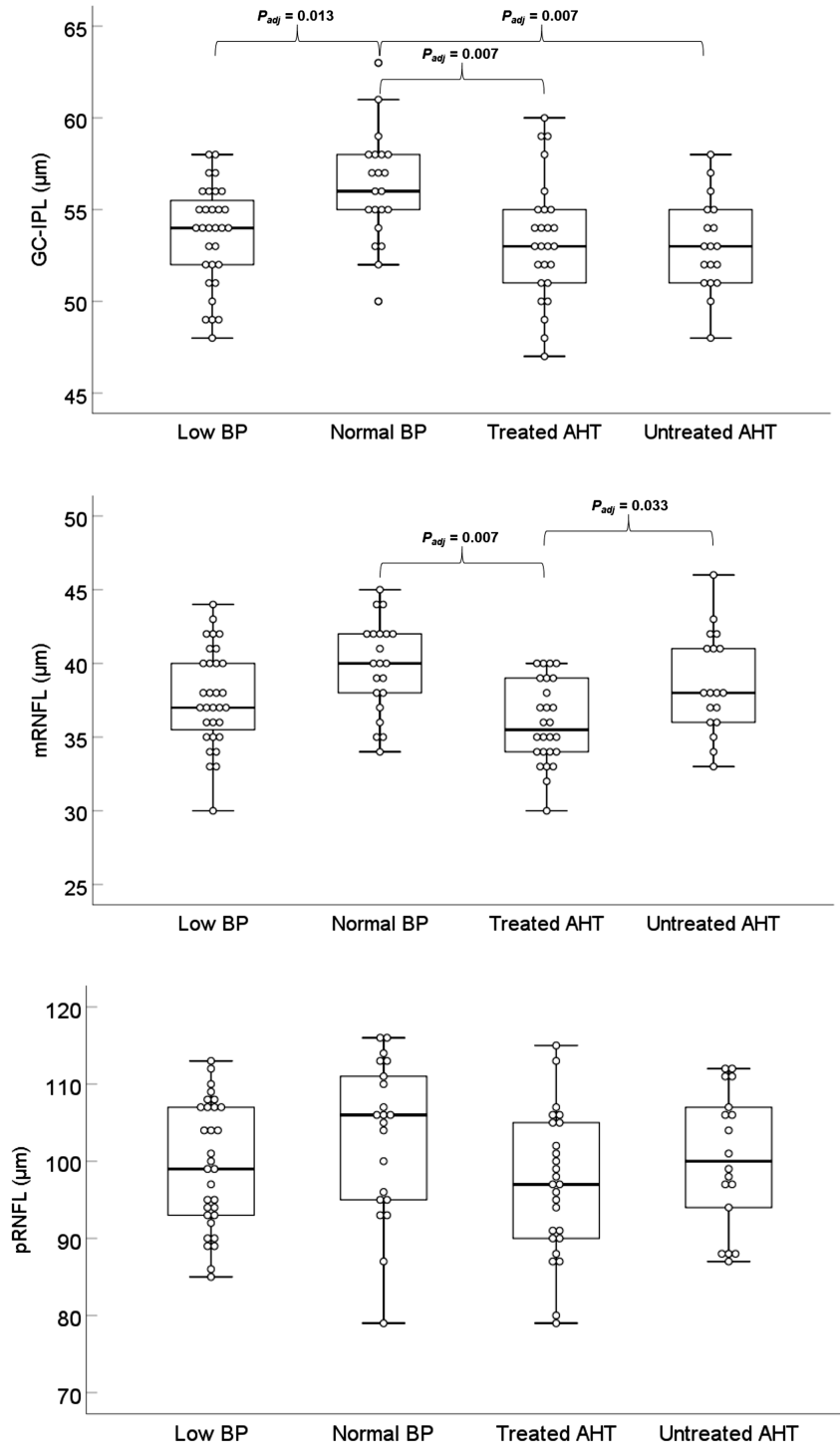
FIGURE 3. GCIPL, mRNFL, and pRNFL as a function of BP status. Significant differences after post hoc comparisons (adjusted for multiple testing) are marked. The thicker layers observed in the control group (normal BP) than in the low BP or AHT groups create a characteristic (inverse) U shape.

with intensively treated arterial hypertension. In the last part of the analysis (mediation analysis) we showed that this compromised capacity for retinal blood flow regulation explains (mediates) the effect of blood pressure status on the GCIPL.

Low Blood Pressure

This is, to our knowledge, the first study to uncover an association between low BP and thinning of the inner retina in ophthalmologically healthy subjects. This relationship
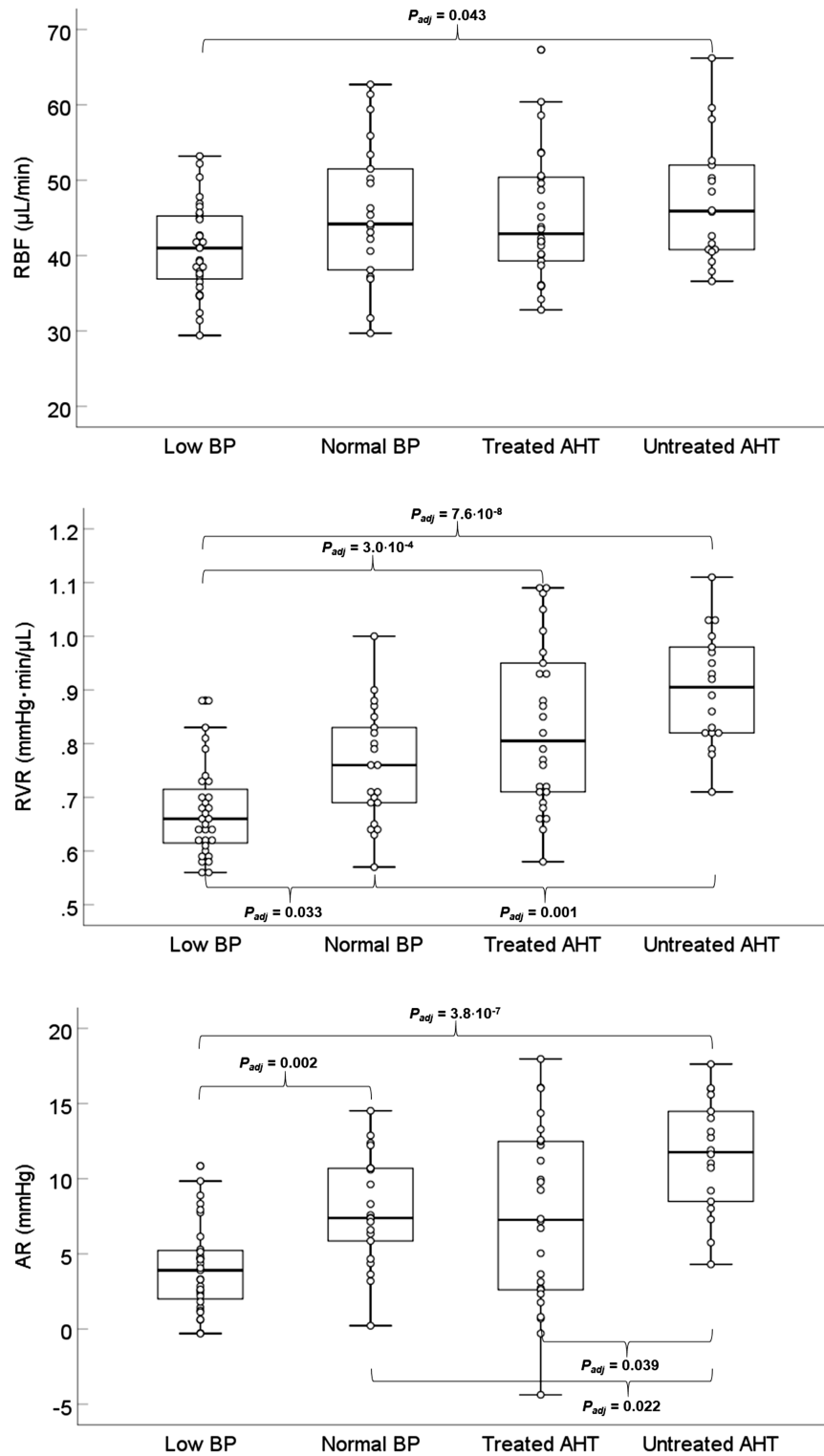
Figure 4. Absolute total RBF, RVR, and AR as a function of BP status. Significant differences after post hoc comparisons (adjusted for multiple testing) are marked. With increasing BP, RBF increases less than RVR, resulting in an autoregulation effect visible in the *first panel*. A statistically significant larger variability is observed for the AR of treated hypertensives (*third panel*), suggesting that subjects in this group can be very close or very far from the lower autoregulation limit.

and its vascular mediation were more pronounced for the GCIPL, which has been shown to be the main layer of early NTG manifestation.[46] In addition, the association was entirely mediated by RBF (no effect of sex, BMI, or other confounders in our population).

Indeed, we have previously shown that LARL for subjects without AHT corresponds to a realistic SBP/DBP of ~105/65 mmHg (or even higher if IOP is above average).[38] Because, in the present study, the average BP reading for the low BP group was at 106/66 mm Hg, our finding that this group had

TABLE 3. Components Used in the Calculation of Vascular Outcomes, as a Function of BP Status

	Group 1 (Low BP)	Group 2 (Normal BP)	Group 3 (Treated AHT)	Group 4 (Untreated AHT)	*P* Value
RPP (mm Hg), mean (SD)	27.2 (2.9)	33.8 (4.1)	36.6 (4.7)	42.0 (4.9)	**<0.001**
Hematocrit,* mean (SD)	0.408 (0.025)	0.428 (0.025)	0.427 (0.026)	0.441 (0.025)	**<0.001**
CRAE (μm), mean (SD)	172 (12)	162 (12)	154 (13)	147 (7)	**<0.001**
CRVE (μm), mean (SD)	228 (17)	228 (16)	229 (18)	222 (12)	0.49
OCT-A FD, mean (SD)	1.626 (0.005)	1.626 (0.007)	1.625 (0.006)	1.626 (0.006)	0.85

* Data from Lifelines. Individualized values adjusted for age, sex, and BP status were used.
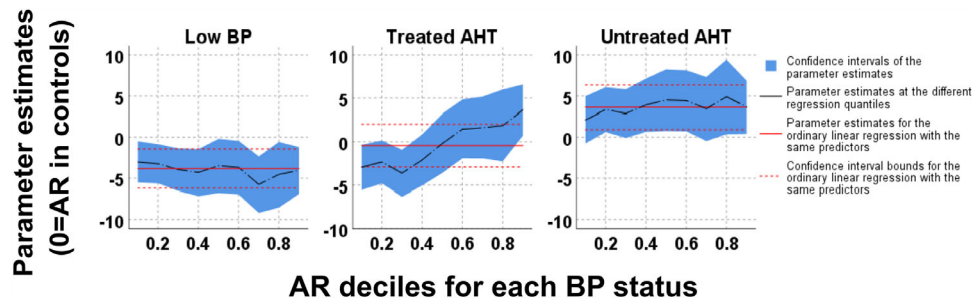

FIGURE 5. Quantile regression models for AR at each BP status. Parameter estimates (y-axis) represent the relative AR (compared to controls) for every AR decile (x-axis). Low BP individuals (*left panel*) have a smaller AR than controls (y < 0), whereas individuals with untreated AHT (*right panel*) have a larger AR than controls (y > 0). Individuals with treated AHT (*middle panel*) have a smaller AR only when intensively treated (leftmost deciles).

a borderline lower RBF (Fig. 4, Table 4) and a considerably smaller AR (Fig. 5, Supplementary Table S3) than controls is in line with our estimations and the general concept of autoregulation.

Population studies have failed to report this association between low BP and GCIPL thickness, possibly because of the implementation of linear models, but an explanation due to differences in genetic background cannot be excluded.[33,34] However, nonlinear models were used is studies with glaucoma as the outcome measure and, in line

with our findings, there is evidence for the existence of increased glaucoma risk with low, usually diastolic or nocturnal, BP.[7–10,12,47,48] It is unknown why low DBP appears to be more frequently associated with glaucoma risk than low SBP.[9,10,12,47] In a study linking nocturnal DBP dips with glaucoma progression, Kwon et al.[49] argued that DBP might better reflect retinal tissue perfusion, which mainly occurs during diastole. That said, this finding is not consistent and could be ethnicity-dependent, because the Barbados Eye Study and the Early Manifest Glaucoma Trial (EMGT)

TABLE 4. Effect of BP Status on GCIPL: Mediation Analysis

	Controls + Low BP (Group 2 + Group 1)		Controls + Treated AHT (Group 2 + Group 3)		Controls + Untreated AHT (Group 2 + Group 4)	
	Effect	*P*	Effect	*P*	Effect	*P*
Mediation Step 1						
GCIPL ∼ MAP	$b_{MAP} = 0.09$	0.052	$b_{MAP} = -0.10$	**0.043**	$b_{MAP} = -0.07$	**0.032**
Mediation Step 2						
RBF ∼ MAP	$b_{MAP} = 0.27$	**0.020**				
RVR ∼ MAP			$b_{MAP} = 0.005$	**0.008**	$b_{MAP} = 0.005$	**0.0003**
AR ∼ MAP			$b_{MAP} = 0.17$	**0.015**		
Mediation Step 3						
GCIPL ∼ MAP + RBF	$b_{MAP} = 0.05$	0.30				
	$b_{RBF} = 0.16$	**0.003**				
GCIPL ∼ MAP + RVR			$b_{MAP} = -0.04$	0.42	$b_{MAP} = -0.03$	0.51
			$b_{RVR} = -11.87$	**0.001**	$b_{RVR} = -10.23$	**0.022**
GCIPL ∼ MAP + AR			$b_{MAP} = -0.06$	0.21		
			$b_{AR} = -0.21$	**0.042**		
GCIPL ∼ MAP + RVR + AR			$b_{MAP} = -0.04$	0.40		
			$b_{RVR} = -27.37$	**0.001**		
			$b_{AR} = 0.47$	**0.032**		
Sobel test	0.059		RVR: **0.041**; AR: 0.095		**0.031**	

reported that lower baseline SBP increases the risk for glaucoma incidence or progression.[7,8] In our study, daytime mean RBF could not account for GCIPL thinning associated with low DBP, further pointing towards the need to study the specific properties of blood flow during diastole or during DBP dips.

An intriguing observation is that some studies that use BP binning report unfavorable glaucoma outcomes already manifesting at a population level at apparently BP normal values (e.g., SBP ≤ 125 mm Hg in the EMGT or the first quartile of DBP in the Singapore Malay Eye Study).[7,9] The Los Angeles Latino Eye study, found that a trend for increased glaucoma prevalence starts at DBP ≤ 71 mm Hg, but reported strong evidence only for DBP ≤ 61 mmHg, which corresponds approximately to the fifth DBP percentile in our population.[10] In our study, we used two different BP binning definitions: one using prospective recruitment criteria based on multiple previous measurements consistently outside the first and ninth BP deciles and one using the standard, cross-sectional fifth and ninety-fifth BP percentile cutoffs. Results were insensitive to the definition chosen and therefore could help interpret the findings of these population studies.

An interesting, albeit not unexpected, observation regarding the low BP group is the fact that it predominantly comprises females whose BMI is on the lower side. This description matches the central traits of a particular phenotype, sometimes referred to as "Flammer syndrome," which has been shown to be associated with NTG, likely owing to deficient RBF or vascular dysregulation.[50]

As also expected, the same group was characterized, on a population basis, by lower hematocrit values. Hematocrit affects blood viscosity, which, in turn, affects RVR and RBF, something that has been accounted for and validated in our estimations.[38,51] Not accounting for the correlation of BP with blood viscosity would erroneously underestimate RBF in the low BP group and overestimate RBF in the hypertensive groups. As such, the viscosity-related indirect effect on structural OCT measurements is actually incorporated in the complete vascular mediation effect presented in Table 4. It is a matter of debate in cardiovascular medicine whether BP is actively modified to compensate for this general effect of hematocrit on organ blood flow.[52] Hyperviscosity has been linked in the past to increased glaucoma risk, but, in our study, we did not find a protective effect of low hematocrit for the low BP group.[53–56] We hypothesize that, due to the stratification of the groups according to BP status and the range of BP examined, BP is the dominant player in our population and a protective effect of hypoviscosity, if any, is masked by the detrimental effect of low BP. Future studies with appropriate, controlled design are needed to elucidate this ambiguity.

Hypertension

Despite a slightly higher RBF and a considerably higher AR (Figs. 4 and 5), untreated hypertensives had thinner GCIPL, which has also been shown to be the location of first progression in glaucoma patients with AHT.[46] This was mediated by RVR (Table 4), that is, the negative effect of increased BP to the GCIPL is explained by increased vascular resistance (but not by reduced blood flow—see below). Nevertheless, it was the treated AHT group that exhibited the most pronounced thinning and this was present in the majority of structural OCT metrics (GCIPL, mRNFL, temporal pRNFL, inferior pRNFL). For this group, GCIPL thinning was independently mediated by RVR and AR (Fig. 5, Table 4). In the univariable models, large RVR and large AR were both associated with thinner GCIPL, because of substantial covariance. After controlling for the confounding effect of RVR, small AR was associated with thinner GCIPL. This suggests that, in treated AHT, a combination of increased resistance and being close to the autoregulatory tipping point explain the negative effect to the GCIPL.

GCIPL thinning without a decrease in RBF seems counterintuitive. However, even when total RBF is largely unaffected, increased RVR results in increased blood velocity (i.e., reduced transit time), shunting of flow, and reduced capillary density.[57,58] This could affect red blood cell distribution and retinal oxygen extraction. Smaller AR was additionally present in intensively treated subjects (Fig. 4C, Fig. 5, Supplementary Table S3), which could mimic low BP and lead to hypoperfusion of the RGCs. In this regard, our results reflect a possible effect of the combined rightward shift of the autoregulation curve (because of atherosclerosis and arteriosclerosis) and the variations of the measured RPP (Fig. 2), due to BP fluctuations throughout the day. Last, chronic AHT also results in endothelial dysfunction and, therefore, impaired autoregulation.[59]

A number of previous studies have also shown an effect of AHT on GCIPL and RNFL thickness.[15,29–32] However, some population-based studies did not detect this relationship.[33,34] Again, study design and analysis methods seem to be the most likely explanations for this discrepancy. Regarding glaucoma risk, the role of AHT is controversial. Most evidence points toward at least some benefit of timely AHT treatment, possibly because of the prevention of microvascular damage, in combination with a slight IOP lowering.[18,60] However, it has been suggested that aggressive treatment of AHT, resulting in low DBP, could negatively affect glaucoma, and we observed a structural effect with the same direction in this healthy population.[14] The confounding contribution of individual antihypertensive medications, whether neuroprotective or detrimental, remains inconclusive.[15–19]

In all, the existence of thinner GCIPL in both low BP and AHT creates the characteristic inverse U-shaped association. Although, theoretically, structural thinning could be attributed to thinner and sparser vasculature contributing to the OCT layer segmentation, it is highly unlikely that this U-shaped association is artificial for two reasons. First, the low BP group had significantly lower RVR, because of significantly broader vascular caliber. This would have led to overestimation, if anything, rather than underestimation of structural metrics. Therefore caliber-related segmentation inaccuracies cannot explain this U-shaped association. Second, associations remained identical before and after compensating for these anatomic confounders in a recent study on antihypertensive medication.[15] The effect of image magnification is also likely negligible, because only mildly ametropic eyes (−3D to +3D, see *Study design and population*) were included, and SEQ was similar between groups. Indeed, using the SEQ values reported in Table 1, it can be estimated that the error in the observed differences attributed to magnification is 0.6% to 1.5%.[61] SEQ was also never significant when adjusted for as a potential confounder, further corroborating this claim.

Study Strengths and Limitations

The main strength of this study was the strict selection process that allowed us to look at the true extremes of

BP. This reduces the noise that usually characterizes larger population studies and results in indirect loss of power. In addition, our linearity-free assumptions and the categorizing of BP (rather than considering it as a continuous variable) allowed us to differentiate between BP status and uncover a U-shaped association that was previously elusive. Last, to our knowledge, this study is the first to provide a rigorous explanation of the differential effect of BP status on retinal structure, by directly linking it to total RBF and its autoregulation.

With regard to limitations, error is certainly associated with RBF, RVR, and AR estimations in the second and third part of our study. In the absence of a gold standard way to quantify these variables, it is difficult to predict the deviation from their actual values. Overall, the physiological component of these variables can be seen in our results, and the range of values we report is in very good agreement with that reported in Doppler OCT studies.[62–67] In addition, we have previously shown, in an independent population, that these outcomes strongly correlate with in vivo blood flow metrics, as assessed by LSFG.[38] Compared to Doppler OCT or LSFG, our approach has the advantage of using more reproducible imaging techniques to quantify vascular caliber and, most importantly, allows for estimation of autoregulation limits. However, it is likely inferior in estimating blood velocity, because velocities are inferred from the calculation of pressures, calibers, viscosities, and branching complexities (see Table 3), rather than directly measured. Combining these methods could therefore further finetune estimations.

It should be also noted that our approach provides information on the effect of static RBF autoregulation, but it is possible that BP status also results in impairment of the autoregulatory latency, that is, dynamic autoregulation, which our study cannot evaluate. As such, our results might only be part of a bigger underlying effect. Similarly, the absence of 24-hour BP monitoring could also result in underestimation of the true effect, because, as already mentioned, individuals in the risk groups might also be prone to nocturnal hypotension.[12] We postulate that these unobserved variables might explain why structural differences were more pronounced than differences in RBF. Because of the cross-sectional nature of our study, absence of data on the first occurrence of AHT is a limitation. However, our threshold of an at least one-year-old diagnosis, together with the selection procedure using multiple previous visits from another database, ensured no newly-diagnosed cases (almost all cases had been diagnosed before at least three years). Last, our population was predominantly Caucasian; it is to be determined whether the results can be generalized to other ethnicities.

Further Considerations

From a theoretical standpoint, there exists a point in the predisease time course when the very first vascular deficits or the very first structural deficits manifest. A subsequent causal cascade of events would then result in further mutually mediated vascular and structural deterioration, sometimes leading to a glaucoma diagnosis. In this regard, one novelty of this study lies in demonstrating that interdependent structural and vascular deficits related to a long-debated cardiovascular risk factor (especially low BP) can even be traced back to whom we perceive as ophthalmologically healthy subjects. Therefore, although this cross-sectional study cannot fully resolve the "chicken-egg" dilemma (we showed that vascular deficits are present without glaucoma, but not necessarily without smaller structural deficits), it provides evidence toward a possible pathophysiological mechanism that warrants further investigation.

Related to that, a similar mediation analysis approach has revealed that structural deficits mediate, in turn, the effect of vascular deficits on glaucoma risk itself.[68] Interestingly, the structural deficits that we report were much more prominent on the macular OCT scans than the ONH scans. It is possible that the initial spatial manifestation of glaucomatous-like damage attributed to vascular factors (NTG phenotype) differs from that of high-tension glaucoma.[46] However, the subject matter is much more complex, because it is beyond doubt that the pathology of NTG itself is still highly dependent on IOP levels, and thus vascular etiology is likely only secondary.[69] Therefore, with the existing evidence, vascular factors should be regarded as additional risk factors, rather than primary driving forces in glaucomatous pathogenesis. The key question for future research is which vascular outcomes can identify increased vulnerability to structural/functional deficit onset and progression.

We would like to stress here that, because there are benefits to intensive BP control with regard to cardiovascular disease, our results should only be seen as such and should not be considered as a case for milder treatment of AHT in general.[70,71] However, because a J- or U-shaped effect is reported in both fields when intensive treatment becomes too intensive, it could be a starting point for discussion with cardiologists in individual cases where, for example, glaucoma continues to deteriorate despite adequate IOP control.[72]

In conclusion, on examination of structural metrics, we uncovered a previously elusive, inverse U-shaped thinning of the GCIPL and RNFL associated with both tails of the blood pressure distribution and with intensive treatment of arterial hypertension, in ophthalmologically healthy individuals. Upon subsequent examination of vascular metrics, we additionally found that GCIPL thinning was differentially associated with reduced retinal blood flow, increased vascular resistance, or insufficient static autoregulatory capacity, depending on blood pressure status. It remains to be seen whether these defects could explain the recurring epidemiological finding of increased glaucoma risk in certain population subgroups, especially subjects with nocturnal blood pressure dipping or intensively treated arterial hypertension. Longitudinal studies are needed to examine this postulation.

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