The Impact of Axial Eye Growth on Foveal Avascular Zone Measurements in Children

Rachel E. Linderman, PhD,[1] Elizabeth Heffernan,[1] Samantha Ferrante, MD,[2] Jane Bachman Groth, OD,[1] and Joseph Carroll, PhD[1,3]*

SIGNIFICANCE: Foveal avascular zone (FAZ) area is a frequently used biomarker in diseases impacting the retinal vasculature in pediatric populations. Variation in axial length between individuals results in differences in lateral image scale, which affect the accuracy of FAZ area measurements. Accordingly, changes in axial length over time within individual children would affect estimates of FAZ area change.

PURPOSE: This study aimed to quantify how changes in axial length over time affect estimates of FAZ area change using optical coherence tomography angiography (OCT-A) images.

METHODS: Twenty pediatric participants (<18 years old) and 40 adult participants were imaged on Optovue's Avanti system (Fremont, CA) and had axial length measurements acquired at two time points. The FAZ was segmented twice using the OCT-A image at each time point. Foveal avascular zone area was estimated at both time points using the assumed/fixed axial length of the OCT-A device (unscaled) and using the participant's axial length (scaled). Changes in FAZ area over time were compared between the pediatric and adult groups using both unscaled and scaled data.

RESULTS: The average ± standard deviation follow-up time was 3.35 ± 1.66 years for the pediatric group and 2.90 ± 1.65 years for the adult group. Using unscaled data, FAZ area seemed to decrease between visits in the pediatric group ($P = .004$), whereas the FAZ area increased between visits in the adult group ($P = .003$). When correctly scaled data were used, the FAZ area still increased between visits for the adult group ($P < .001$), although the FAZ area no longer showed a significant change between visits for the pediatric group ($P = .37$). When comparing the normalized FAZ area change across visits between unscaled and scaled data, a significant difference was found between the adult and pediatric groups ($P < .001$).

CONCLUSIONS: Scaled data should be used when measuring FAZ area in pediatric populations, especially in longitudinal studies.

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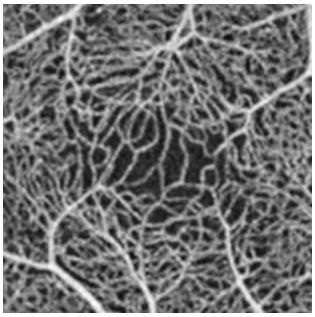

Author Affiliations:
[1]Department of Ophthalmology and Visual Sciences, Medical College of Wisconsin, Milwaukee, Wisconsin
[2]School of Medicine, Medical College of Wisconsin, Milwaukee, Wisconsin
[3]Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, Wisconsin
*jcarroll@mcw.edu

Optical coherence tomography angiography (OCT-A) is used routinely to image the retinal vasculature including the foveal avascular zone. The morphometry of the foveal avascular zone is used as a biomarker in various diseases and conditions in children and adolescents. For example, children born prematurely have been shown to have reduced or absent foveal avascular zones,[1–3] whereas children with sickle cell disease have been shown to have larger foveal avascular zone areas compared with controls.[4] In addition, children with diabetes type 1 have been shown to have significantly different foveal avascular zone perimeters before clinically significant diabetic retinopathy or changes in foveal avascular zone area.[5,6] Optical coherence tomography angiography has also been used to examine pediatric populations afflicted with other diseases like radiation retinopathy,[7] obstructive sleep apnea-hypopnea syndrome,[8] and amblyopia.[9] The use of OCT-A in pediatric populations (including neonates) is expected to rise because of advances in handheld OCT and OCT-A technology.[10–12] With this growing use, the accuracy of foveal avascular zone measurements in children is becoming an increasingly important issue.

Accurate measurements of the foveal avascular zone require knowledge of the lateral scale of the OCT-A image from which they are made. Individual differences in axial length are known to affect the lateral scale of the OCT-A image,[13,14] although many studies do not correct for this in their analyses.[15] Although some imaging devices allow for the input of ocular biometry values at the time of imaging, there have not been systematic studies on the accuracy of these corrections, which is further complicated by different devices using different assumed model eyes. Conclusions from cross-sectional studies comparing foveal avascular zone metrics between groups may not be impacted by the use of incorrect lateral scale, particularly if there are no differences in axial length between the groups. However, such studies may also underreport or overreport variation in the measurements, thus causing the true effect size or changes in foveal avascular zone metrics to be masked. More so than cross-sectional studies, longitudinal studies in pediatric populations are more susceptible to scaling changes, as the eye continues to elongate well into the late teenage years in most individuals.[16–20] Although standard growth curves are available for pediatric and neonatal populations,[12,18] the rate of eye growth is highly variable across individuals,[21] which can reduce the accuracy of scaling adjustments. Without incorporating estimates of the actual image scale

in longitudinal studies, it is impossible to determine whether observed changes in foveal avascular zone morphometry are due to real structural changes in the foveal avascular zone or simply due to use of an incorrect lateral image scale (either from not factoring in eye growth or from the individual having eye growth different from the average observer). This uncertainty confounds studies of normal foveal and/or foveal avascular zone development as well as accurate characterization of disease progression. Here we empirically examined how eye growth impacts foveal avascular zone area measurements over time in children.

METHODS

Participants

We recruited 20 pediatric (younger than 18 years) participants, and 40 adult (18 years and older) participants with no self-reported ocular or systemic diseases. This study was approved by the institutional review board of the Medical College of Wisconsin and was conducted in accordance with the tenets of the Declaration of Helsinki. For participants 18 years and older, informed consent was acquired after discussing the nature and possible consequences of the study. For participants younger than 18 years, permission was obtained from the parent or legal guardian, and assent was obtained from the minor after discussing the study as stated previously.

OCT-A Imaging

One eye from each participant was imaged on Optovue's Avanti system (Fremont, CA) at two time points. Two to four macular volume scans measuring 3 × 3 mm (nominal dimensions) were acquired at each time point. Each volume comprises two separate scans, one acquired in the fast-x direction and one in the fast-y direction. The scans are registered by the system software (version 2018.1.0.43) to generate a single volume for analysis. An angiogram from each volume was generated (inner limiting membrane to 9 µm above the outer plexiform layer), and the best angiogram (subjectively determined by the first author, REL) per visit for the selected eye was exported for further analysis. Image quality assessment considered motion artifacts, signal-to-noise ratio, and visibility of retinal vasculature with an emphasis on the foveal area. The axial length of each eye was measured at both time points using an IOLMaster 500 (Carl Zeiss Meditec, Dublin, CA), which uses multiple individual A-scan measures to derive an estimate of axial length.

Image and Statistical Analysis

Using ImageJ's multipoint tool,[22] a single masked observer (second author, EH) segmented the foveal avascular zone in each image twice. After this, two reviewers (authors REL and JC) reviewed the segmentations for errors. If an error was found (n = 21 for trial 1, n = 22 for trial 2), one reviewer (author REL) then examined the unmarked images and single-volume images in the fast-x direction (if needed) to find the subjectively best segmentation so that the errors could be corrected. The segmentation was corrected at the location of the error on the marked image (leaving the rest of the original segmentation unchanged). Once all of the segmentations were deemed accurate, we used the MATLAB (MathWorks, Natick, MA) function *polyarea* to calculate the area in pixels within the (x, y) segmentation coordinates.[23] For each segmentation, the area in pixels was converted to retinal area in micrometers using a constant nominal image scale of 9.87 µm/pixel (nominal image scan width, 3 mm; image size, 304 × 304 pixels), which we will refer to as the unscaled data. To generate the scaled area values in micrometers, we used a different scaling factor for each subject. This was calculated by multiplying the nominal scaling factor (9.67 µm/pixel) by the ratio of the subject's measured axial length to the assumed model eye length of the device (23.95 mm).

The repeatability and the reliability (R Foundation for Statistical Computing, Vienna, Austria[24]) between the two segmentations and between the axial length measurements were examined. A linear regression examining the rate of change for axial length based on the average age of all individuals was calculated (GraphPad Prism 9; GraphPad Software, San Diego, CA). The difference in average foveal avascular zone area between visits was calculated for both sets of data and the rate of change. Comparisons between the time points for each set of data, between data sets, and the rate of change for foveal avascular zone area were tested using either an unpaired t test or the Mann-Whitney U test (GraphPad Prism 9). The selection of the test used was dependent on an analysis of normality ($P < .05$) using the Shapiro-Wilk normality test. Finally, the change in foveal avascular zone area between visits for the scaled and the unscaled data was calculated and then normalized to the first visit's measurements. The change in the foveal avascular zone area measurements was then categorized as either positive or negative for both the unscaled and scaled data, and the odds ratios for the adult and pediatric groups were calculated while controlling for which group the participants were in using the Cochran-Mantel-Haenszel test using R (R Foundation for Statistical Computing[24]).

RESULTS

Demographic details about the participants and imaging time points are found in Table 1 for the pediatric group and Table 2 for the adult group. Overall, the average ± standard deviation (SD) age of all participants was 28.56 ± 17.23 years with 46 females (77%). There was no significant difference in the average ± SD follow-up time between the pediatric participants (3.35 ± 1.66 years) and the adult participants (2.90 ± 1.65 years; Mann-Whitney U test, $P = .41$). We examined the measurement error for our axial length measurements (defined as 1.96 times the within-subject SD, or 1.96 × S_w).[25] Using the multiple measurements for all 60 participants obtained at the first visit, we found a measurement error of 0.04 mm, meaning that the difference between a given measurement and the true value would be less than 0.04 mm for 95% of measurements.[25] As expected, axial length increased between visits for the pediatric group ($P < .001$, Wilcoxon signed rank test) but not the adult group ($P = .26$, paired t test). The average ± SD rate of change for axial length for all participants was 0.03 ± 0.05 mm/y, although when the participants were categorized based on their age, the pediatric group had an average ± SD of 0.08 ± 0.06 mm/y, whereas the adult group had a lower average rate of 0.01 ± 0.03 mm/y. There was a significant relationship between the average age of participants and the rate of axial length change, meaning that, as average age of the participants increased, the change between visits for axial length decreased ($y = –0.002x + 0.08$, $P < .001$).

Although the repeatability of foveal avascular zone measurements has been previously reported,[13,26,27] we examined the repeatability using the specific observer and method used in this study as we had repeated segmentations on all 120 images. The repeatability (defined as 2.77 times the within-subject SD, or 2.77 × S_w) was 0.005 mm^2 (or 1.9% of the average scaled foveal avascular zone area). This

TABLE 1. Demographics and scaled FAZ area data for the pediatric participants

		1st visit			2nd visit		
Subject	**Sex**	**Age (y)**	**Axial length (mm)**	**FAZ area (mm^2)**	**Years since 1st visit**	**Axial length (mm)**	**FAZ area (mm^2)**
JC_11336	F	5.4	22.72	0.249	3.23	23.16	0.250
JC_11863	M	6.6	23.00	0.469	0.86	23.13	0.472
JC_10719	F	8.4	23.02	0.222	5.02	23.26	0.215*
TD_11263	F	8.8	22.96	0.300	2.48	23.14	0.300
JC_10648	M	8.9	21.44	0.197	5.51	21.56	0.202†
JC_0878	F	9.1	23.46	0.314	5.29	24.65	0.333†
JC_12031	F	9.5	23.27	0.056	2.31	23.51	0.052*
JC_11862	F	10.2	22.12	0.534	0.86	22.18	0.525
JC_10690	M	10.5	24.05	0.160	5.03	24.57	0.155*
JC_10712	F	10.6	24.27	0.222	3.96	24.72	0.230†
JC_10649	F	10.7	22.23	0.290	5.51	22.72	0.308†
JC_10684	F	11.3	24.26	0.221	3.78	24.65	0.226†
JC_11910	F	11.6	22.47	0.308	2.31	22.66	0.311
JC_0077	F	11.8	23.77	0.094	5.11	24.35	0.101†
JC_10646	F	12.3	22.82	0.137	5.51	23.02	0.131*
JC_10754	F	12.3	23.59	0.410	2.69	23.90	0.422†
JC_12032	M	12.7	24.67	0.117	2.34	24.61	0.119
JC_11911	M	14.1	23.85	0.313	2.31	23.97	0.316
JC_11598	F	14.2	23.63	0.214	1.41	23.58	0.202*
JC_11597	F	17.7	24.01	0.356	1.41	24.07	0.346*
Summary data	15 F/5 M	10.8 ± 2.8	23.28 ± 0.83	0.259 ± 0.123	3.35 ± 1.66	23.57 ± 0.90	0.261 ± 0.124

*Significant decrease in FAZ area between the two visits (greater than –1.9%). †Significant increase in FAZ area between the two visits (greater than +1.9%). F = female; FAZ = foveal avascular zone; M = male.

means that the difference between two repeated measurements for the same participant would be less than 1.9% for 95% of pairs of observations. In addition, the interclass correlation coefficient was calculated at 0.9997 (confidence interval, 0.9996 to 0.9998). Given the excellent intraobserver repeatability of foveal avascular zone area measurements, the area values from the two segmentations for each image were averaged.

When using the nominal image scale, the foveal avascular zone area in the pediatric group seemed to shrink between imaging sessions by an average ± SD of 0.005 ± 0.006 mm^2 ($P = .004$, paired *t* test; Fig. 1A). When scaling for axial length was done, there was no difference (on average) in foveal avascular zone area between the two visits (average ± SD difference was 0.002 ± 0.009 mm^2; $P = .37$, paired *t* test; Fig. 1B). In contrast, the adult group showed a small but significant increase in foveal avascular zone area between visits both when their images were analyzed using the nominal image scale (average ± SD increase, 0.003 ± 0.007 mm^2; $P = .003$, paired *t* test) and also when their images were scaled using axial length (average ± SD increase, 0.003 ± 0.006 mm^2; $P < .001$, paired *t* test).

When looking at the overall change for foveal avascular zone area between visits, we observed a significant difference between our adult and pediatric groups. In our adult group, we saw no significant difference in the normalized change in foveal avascular zone area between visits when comparing the unscaled (average ± SD, 1.46 ± 2.87%) and scaled data (1.47 ± 2.70%; $P = .76$, Wilcoxon matched-pairs signed rank test; Fig. 2). In contrast, there was a significant difference in the normalized change in foveal avascular zone area between visits for the pediatric group ($P < .001$, Wilcoxon matched-pairs signed rank test). In the pediatric group, the average ± SD change in the foveal avascular zone area between visits was –2.03 ± 3.27% when using the unscaled data, suggesting that the foveal avascular zone decreased in size on average. However, when using the scaled data, the foveal avascular zone area did not significantly change between visits, with the average ± SD change of 0.43 ± 3.99%.

We also assessed the rate of change for each data set to ascertain how the amount of time in between visits impacted the change in foveal avascular zone area. The adult participants did not have a significant difference in the observed rate of change for unscaled (average ± SD, 0.0013 ± 0.0045 mm^2/y) or scaled data (average ± SD, 0.0014 ± 0.0042 mm^2/y; $P = .62$, Wilcoxon matched-pairs signed rank test; Figs. 1C, D). Conversely, there was a significant difference in the rate of change for pediatric participants when comparing the unscaled and scaled data ($P < .001$; Wilcoxon matched-pairs signed rank test). When using the unscaled data, the average ± SD rate of change for the foveal avascular zone area in the pediatric group was –0.0025 ± 0.0042 mm^2/y, meaning the foveal avascular zone area seemed to decrease between visits. However, this shifted closer to 0 when using the scaled data, with the average ± SD rate of change of the foveal avascular zone area being –0.0003 ± 0.0040 mm^2/y.

TABLE 2. Demographics and scaled FAZ area data for the adult participants

		1st visit			2nd visit		
Subject	**Sex**	**Age (y)**	**Axial length (mm)**	**FAZ area (mm^2)**	**Years since 1st visit**	**Axial length (mm)**	**FAZ area (mm^2)**
JC_10803	F	21.3	24.1	0.328	2.00	24.08	0.336*
JC_11409	F	22.1	23.44	0.375	0.99	23.48	0.378
JC_12019	F	23.1	24.02	0.160	1.97	24.11	0.162
JC_12020	F	23.3	22.97	0.068	2.18	22.96	0.073*
JC_0905	M	23.3	22.75	0.278	2.68	22.82	0.280
JC_10586	F	23.7	21.41	0.164	6.11	21.37	0.175*
JC_11441	F	23.9	23.13	0.324	3.14	23.13	0.329
JC_10591	M	24.0	23.62	0.141	6.11	23.55	0.146*
JC_10567	F	24.2	22.26	0.276	5.50	22.33	0.286*
JC_0769	F	24.7	24.48	0.310	5.86	24.43	0.314
JC_11610	M	24.7	23.95	0.079	3.32	23.97	0.079
JC_10578	F	25.4	24.33	0.272	2.62	24.44	0.278*
JC_11444	F	26.3	24.00	0.383	3.21	24.00	0.385
JC_10990	F	26.7	24.70	0.225	3.79	24.68	0.220†
JC_10777	F	27.7	26.04	0.240	1.20	26.00	0.252*
JC_0200	M	28.0	24.56	0.224	6.06	24.46	0.227
JC_11159	F	28.6	23.64	0.260	3.01	23.63	0.257
JC_11295	F	29.6	22.88	0.464	1.27	22.91	0.470
JC_11321	F	29.7	23.67	0.364	1.15	23.75	0.364
JC_11068	F	31.5	22.39	0.195	1.02	22.43	0.193
JC_0617	M	31.8	23.73	0.061	4.33	23.73	0.063*
JC_11335	F	33.5	23.84	0.220	3.23	23.81	0.223
JC_10691	F	38.7	24.39	0.463	2.72	24.59	0.465
JC_10575	F	39.9	24.63	0.105	5.11	24.63	0.107*
JC_10692	M	40.0	23.70	0.322	2.72	23.68	0.336*
JC_0007	M	40.2	27.44	0.077	3.41	27.58	0.078
JC_10650	F	41.7	23.38	0.258	5.51	23.30	0.272*
JC_10673	F	42.7	22.09	0.184	0.90	22.08	0.196*
JC_0691	F	46.8	26.45	0.316	3.70	26.45	0.323*
JC_1103	F	47.8	26.70	0.331	2.27	26.83	0.339*
JC_11102	M	49.1	24.94	0.053	1.99	24.93	0.050†
JC_10145	F	50.0	24.66	0.279	3.44	24.72	0.265†
JC_11538	F	51.7	24.05	0.364	0.94	24.14	0.367
JC_11103	F	53.7	24.59	0.264	2.91	24.42	0.265
JC_11086	F	59.9	25.90	0.286	2.97	25.84	0.285
JC_11467	F	60.8	23.60	0.230	1.30	23.62	0.231
JC_11468	F	62.6	23.92	0.373	0.56	23.91	0.368
JC_10584	F	63.2	23.13	0.285	1.16	23.12	0.301*
JC_11661	M	64.2	23.65	0.221	0.67	23.66	0.215†
JC_10705	F	66.8	24.38	0.424	2.97	24.44	0.429
Summary data	31 F 9 M	37.4 ± 14.3	24.04 ± 1.23	0.256 ± 0.108	2.90 ± 1.65	24.05 ± 1.24	0.260 ± 0.109

*Significant increase in FAZ area between the two visits (greater than +1.9%). †Significant decrease in FAZ area between the two visits (greater than −1.9%). F = female; FAZ = foveal avascular zone; M = male.

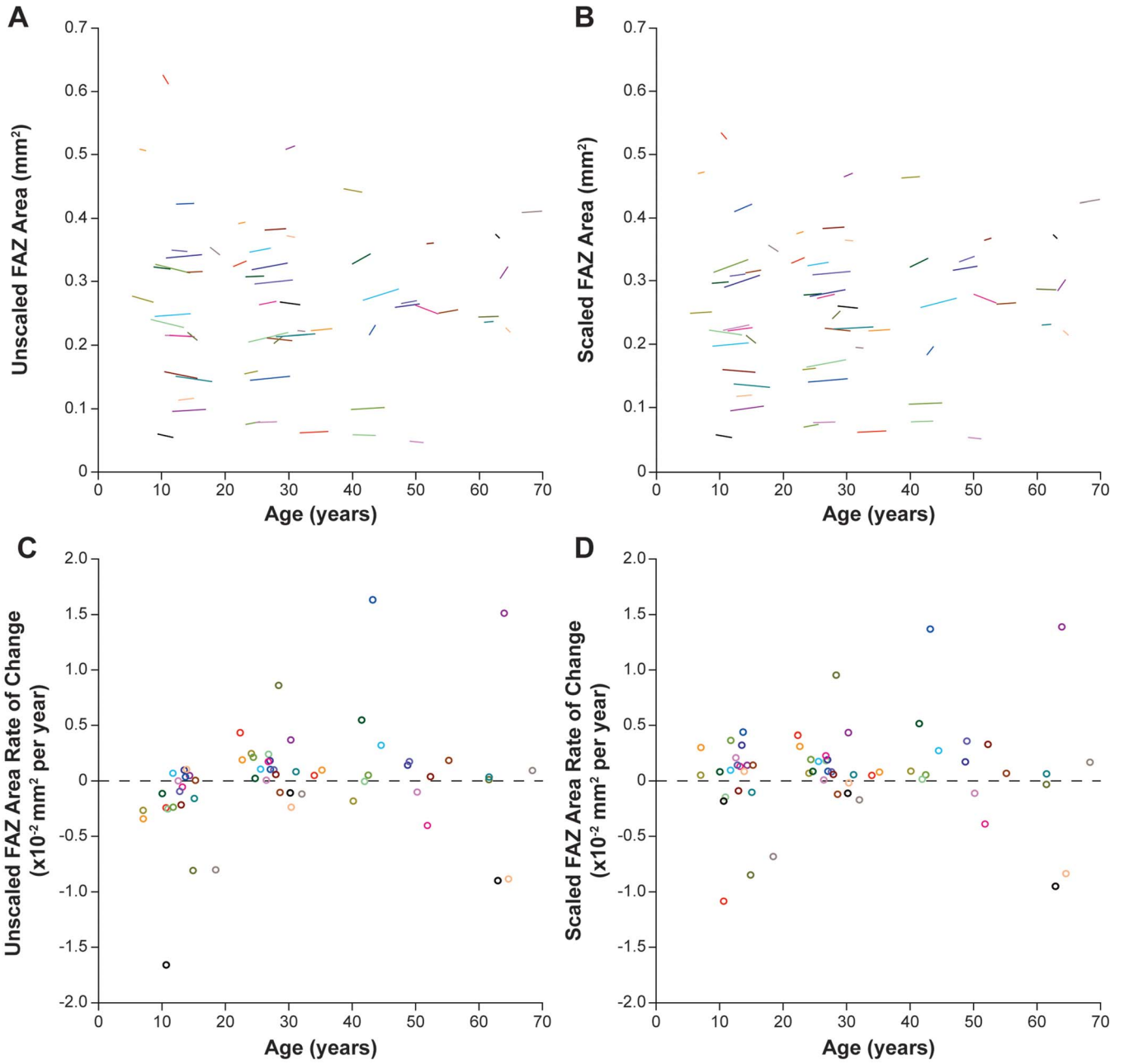

FIGURE 1. Parametric and change in foveal avascular zone (FAZ) area data for unscaled and scaled. (A) Parametric data for all participants with the FAZ measured using unscaled data. (B) Parametric data for all participants with the FAZ measured using scaled data. When we examine the rate of change for FAZ area in our adult participants, there was no significant differences in the rate of change for the FAZ area when using the unscaled (C) and scaled (D) data (Wilcoxon matched-pairs signed rank test, $P = .63$). However, when we examined our pediatric participants, there was a significant difference in the rate of change when comparing unscaled and scaled data (Wilcoxon matched-pairs signed rank test, $P < .001$).

We also categorically (increase, decrease, no change) examined changes in foveal avascular zone area between visits for both unscaled and scaled data (Fig. 3). Based on our intraobserver repeatability data, we used 1.9% as the threshold of "real" change when examining the difference in foveal avascular zone area between visits for individual participants (changes less than this were not considered significant). For the foveal avascular zone area measurements between visits in the 20 pediatric participants, we observed a decrease in 9 participants (45%), an increase in 2 participants (10%), and no change in 9 participants (45%) using the unscaled data (Fig. 3A). When using scaled data, we observed a decrease in 6 participants (30%), an increase in 7 participants (35%), and no change in 7 participants (35%; Fig. 3B). It is worth noting that 45% of the pediatric participants switched categories when using unscaled versus scaled data. All but one of these switches resulted in a shift to more positive change over time (i.e., went from a decrease in foveal avascular zone area to no change, a decrease in foveal avascular zone area to an increase in foveal avascular zone area, or no change to an

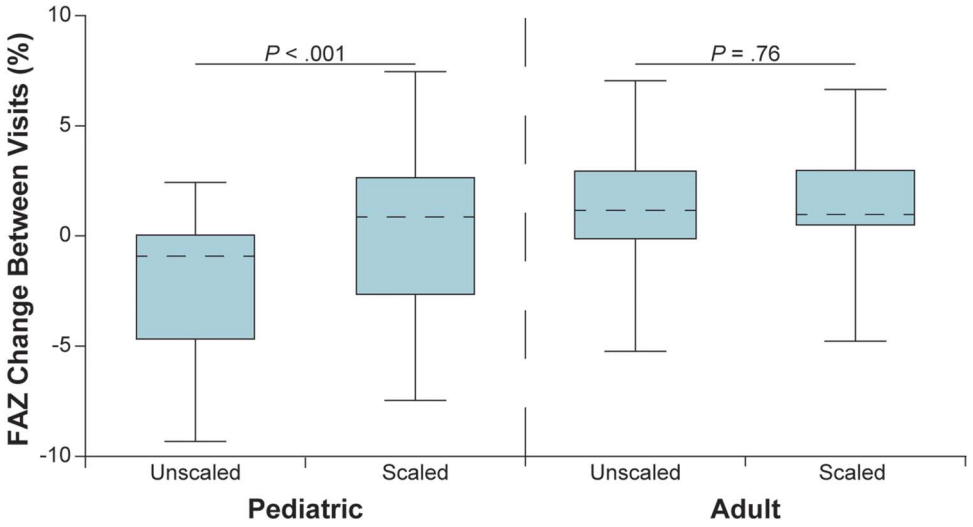

FIGURE 2. Comparison between unscaled and scaled data when measuring the change in foveal avascular zone (FAZ) area between visits. Change in FAZ area was normalized as a percent of the FAZ area at the first time point for each subject in both the pediatric and adult groups (dashed lines, mean change; box limits, 25th to 75th percentiles; whiskers, minimum and maximum change). There was a significant difference between the unscaled and scaled data for the pediatric group when looking at the change in normalized FAZ area between visits. In contrast, there was no significant difference between the unscaled and scaled data when looking at the change in normalized in FAZ area between visits in the adult group.

increase in foveal avascular zone area), which is to be expected given that the axial length (and thus the image scale in micrometers per pixel) was increasing between visits in all but two pediatric participants. The largest difference was seen in JC_0878 who had a 3.9% decrease in foveal avascular zone area when using unscaled images but a 6.1% increase in foveal avascular zone area when using their scaled data.

In contrast, only six (15%) of the adult participants switched categories when using unscaled versus scaled data. Unlike the pediatric group, the majority (n = 4; 66%) of these were a shift to more negative change over time (an increase in foveal avascular zone area with unscaled data to no change with scaled data). When using unscaled data, we observed a decrease in 4 participants (10%), an increase in 17 participants (42.5%), and no change in 19 participants (47.5%; Fig. 3C). When using scaled data, we observed a decrease in 4 participants (10%), an increase in 15 participants (37.5%), and no change in 21 participants (52.5%; Fig. 3D). Accordingly, there was a significant difference in the odds ratios between the two groups ($P < .001$, Cochran-Mantel-Haenszel test), meaning that change in slope between the scaled and unscaled data is not independent of which group the participant is in. This is consistent with image scaling being more critical in pediatric versus adult populations when trying to assess the direction and magnitude of change in foveal avascular zone area over time.

DISCUSSION

Optical coherence tomography angiography is a widely used, noninvasive method to assess the retinal vasculature. The ability to easily visualize and quantify features like the foveal avascular zone allows physicians and researchers to follow progression and patterns of the retina in conditions such as sickle cell,[4] diabetes,[5,6] retinopathy of prematurity,[3] and those receiving radiation therapies.[7] With lateral measurements (e.g., foveal avascular zone area) being used as biomarkers, it is important to evaluate factors that affect the accuracy (and thus the potential utility) of these measurements. Here, we observed that, without correct scaling, the foveal avascular zone area seems to shrink with continued eye growth over time in 40% of the pediatric eyes in our study. However, when using images scaled using individual axial length data, only 35% of the pediatric eyes in our study showed a real decrease in foveal avascular zone area over time. In comparison, 42.5% of participants older than 18 years in this study showed an increased foveal avascular zone area between visits using both their scaled and unscaled data.

Previous cross-sectional studies have examined how age impacts different vasculature metrics in pediatric groups both with[28] and without axial length correction.[29–31] These studies found that foveal avascular zone area does not change on average with age, whereas our scaled longitudinal data demonstrated that some participants had an increase in foveal avascular zone area and other participants stayed relatively stable (Table 1), although, on average, there was no significant change over time. There were six pediatric participants whom even with axial length scaling showed a decrease in foveal avascular zone area. One explanation for the increase in foveal avascular zone area could be mechanical stretching of the retina because of eye growth, as there are active remodeling processes at the fovea in the maturing retina.[32]

When we examine our pediatric data without any axial length correction, we did observe a small but significant decrease in the size of the foveal avascular zone on average over time unlike Zhang et al.[28] and Kiziltoprak et al.[30] The inconsistency between studies may be due to the large variability in foveal avascular zone area across the general population, which makes quantifying the impact of age difficult to calculate when using cross-sectional data. Interestingly, our pediatric longitudinal data do agree with the study published by Hsu et al.,[31] which did not correct for axial length and with Li et al.[29] whose study did correct for axial length. Correcting for differences in axial length in studies is still rare mostly because

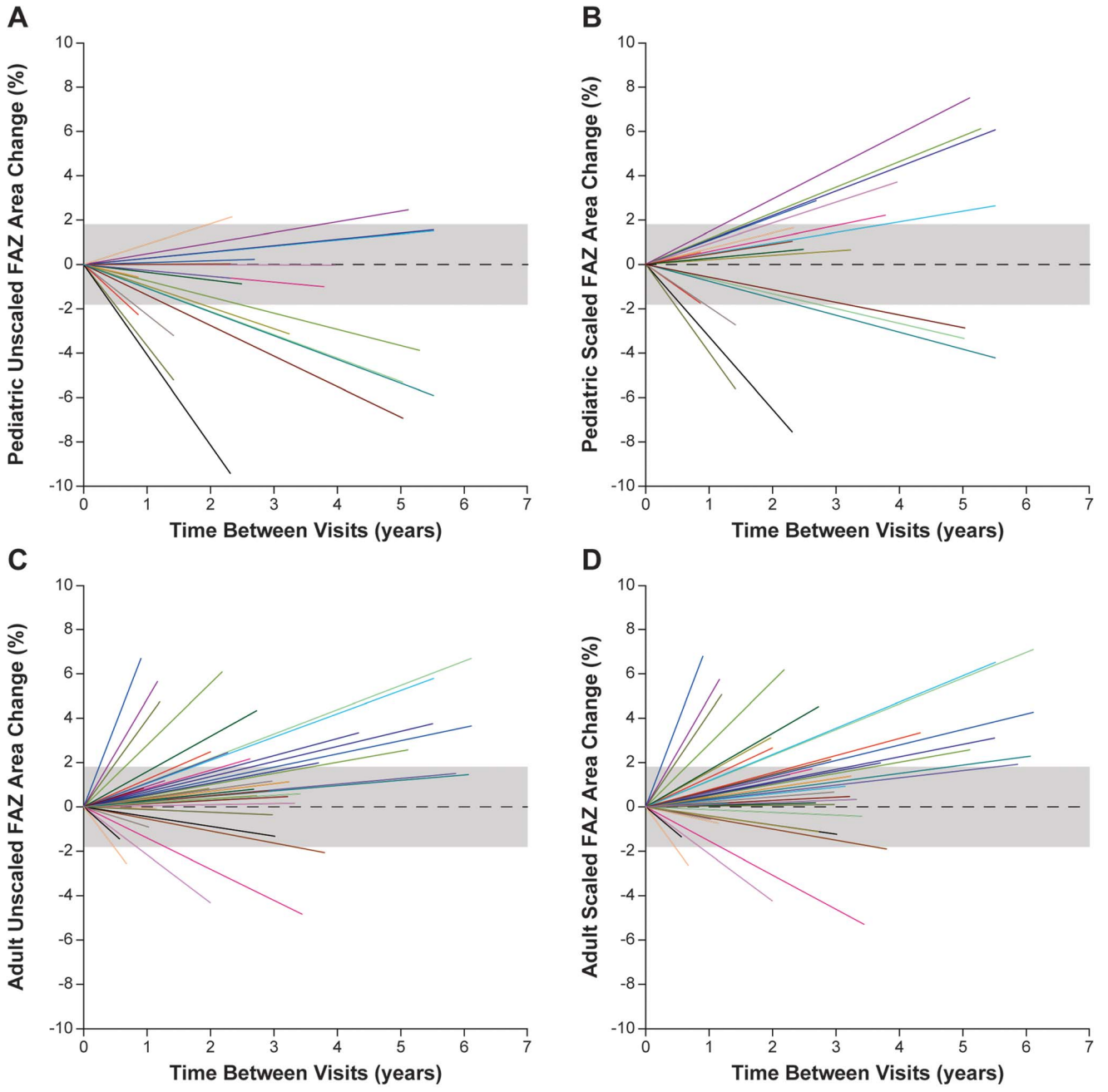

FIGURE 3. Unscaled and scaled data from both the pediatric and adult groups measuring the change in normalized foveal avascular zone (FAZ) area measurements between visits. The shaded gray area marks the range in which changes in FAZ area are within our repeatability value (±1.9%); changes outside of this range were considered real changes in FAZ area. (A) Using unscaled data from the pediatric group, 2 of 20 participants (10%) showed an increase in FAZ area between visits, and 9 participants (45%) showed a decrease in FAZ area between visits. (B) When scaled data were used from the same group, 7 of 20 participants (35%) showed an increase in FAZ area between visits and 6 participants (30%) showed a decrease in FAZ area between visits. (C) When using unscaled data for the adult group, 17 of 40 participants (42.5%) showed an increase in FAZ area between visits, whereas 4 participants (10%) showed a decrease in FAZ area between visits. (D) When using scaled data, the same 4 participants showed a decrease in FAZ area between visits, whereas 15 of 40 participants (37.5%) showed an increase in FAZ area between visits.

of clinical work flow and no automatic ability to adjust lateral scale within most OCT devices.[15] Eye growth models meant to approximate changes in the lateral scale (due to axial length growth) offer a more accessible means to address this issue.[12,18] However, because there is significant individual variation in axial length at all ages, reliance on a growth curve to derive image scale would lead to an overestimation or underestimation in foveal avascular zone area change over time in some individuals (especially in children who develop refractive errors).[16,19]

In our adult group, both the unscaled and scaled data showed a small average increase in foveal avascular zone between visits. This is in agreement with some studies,[33–35] although it disagrees with

others.[36–38] Fujiwara et al.[38] did correct for axial length measurements in their study but found no significant association between foveal avascular zone area and age. This apparent discrepancy could be due to their study being cross-sectional in nature rather than longitudinal—as noted previously, the large normal variability in foveal avascular zone area makes it difficult to detect small age-related differences in cross-sectional studies. When looking at the rate of change, we observed an average increase of 0.0014 ± 0.0042 mm^2/y when using correctly scaled images. This is the same as Iafe et al.[33] and similar to Gong et al.,[35] who reported an increase of 0.001 mm^2/y. There are a number of possible explanations to account for the apparent increase in foveal avascular zone area. First, there could be real changes in the capillaries that define the foveal avascular zone area, which we did observe in one of our adult participants (Fig. 4). In addition, the adult participant with the largest rate of change in foveal avascular zone area (subject JC_10673) showed a change in the shape of their foveal pit between visits (pit depth decreased by 3% and pit volume decreased by 9%). Because the capillaries that define the foveal avascular zone area reside in the inner retinal layers, any deformations or changes in the foveal contour could affect the morphometry of the foveal avascular zone. In a similar regard, age-related thinning of the inner retina has been documented,[39–41] and it is possible that this thinning could also cause outward lateral displacement of the capillary vessels that define the foveal avascular zone. Other explanations relate to the scaling method used. As stated previously, accurate measurements of the foveal avascular zone require knowledge of the lateral scale of the OCT-A image. Previous reports have shown lens thickness increases and anterior chamber depth decreases with age.[42,43] Based on a previously published model,[44] these changes in lens thickness and anterior chamber depth would alter image scale and result in an apparent increase in the foveal avascular zone area. However, the amount of increase induced would be about an order of magnitude less than what we and others have observed.[33,35] The measurement error in axial length could also induce inaccuracies in the measured foveal avascular zone area; however, the measurement error would not be expected to be systematic in one direction, so it is unlikely to account for the increase in foveal avascular zone area between visits. Disambiguating real age-related changes in the foveal avascular zone area versus measurement artifacts is critical to the potential use of foveal avascular zone area as a biomarker in aging and age-related conditions like Alzheimer disease.

Although we looked at foveal avascular zone area, other imaging modalities that acquire images based on a fixed image size would also be impacted. For example, lateral measurements on conventional OCT images such as retinal nerve fiber circle diameter on the optic nerve head or foveal pit diameter would be impacted. In addition, tests focusing on the structure-function relationship of different cell types at various locations in the macula would also be impacted by changes in lateral scale.[45] It is important to note that measurements taken within a set area (such as retinal thickness within the Early Treatment Diabetic Retinopathy Study grid) may not be impacted explicitly, but the comparison between individuals and groups would be compromised because of variable amount of the retinal area contributing to the measurement across individuals. In addition, there are nonuniformities in retinal stretch such that axial length has been shown to impact peripapillary vasculature metrics differently than parafoveal vascular metrics.[46] Finally, because of the shape of the eye, there is a nonlinear effect of axial length on retinal image magnification.[47] Individualized optical models using whole-eye OCT may assist in estimating retinal curvature[48] and thus are the appropriate scaling to apply to images collected outside the foveal region. As widefield retinal imaging becomes more prevalent, such issues will become increasingly important.[49]

There are some important limitations to our study, the first being our relatively small sample size, although the longitudinal design of our study does help compensate for our small number. Although the follow-up time was just more than 3 years on average (and variable across participants), this was sufficient to demonstrate empirically the impact of increasing axial length over time in children on foveal avascular zone area measurements. However, the growth rate estimates may not extend to longer follow-up periods, so continued longitudinal studies are important. Our study population was also greater than 75% female, although there was no difference in the sex distribution of the adult and pediatric groups. Because males have longer eyes on average than females, it would be important to repeat these studies to examine whether the impact/error of eye growth is larger in male children. Finally, our image scaling method only accounted for changes in axial length and did not account for other aspects of ocular biometry (e.g., anterior chamber depth, refraction, and corneal curvature) that have been shown to have a small effect on retinal image magnification.[44] Despite these limitations, we have shown that not accounting for axial length in a pediatric population over time can result in incorrect conclusions regarding both the direction and magnitude of changes in foveal

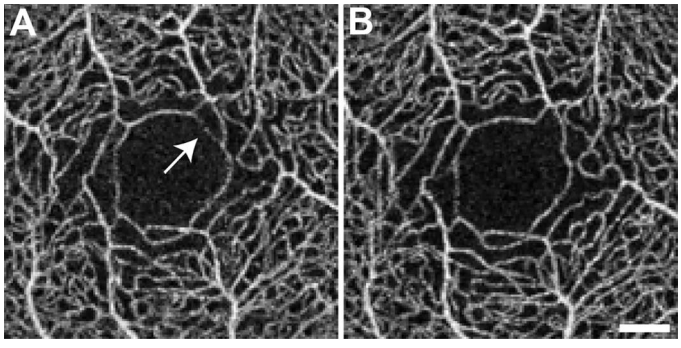

FIGURE 4. Example of a participant where the foveal avascular zone (FAZ) area changed over time. The first image (A) was taken when JC_10586 was 23.7 years old. The second image (B) was taken 6.11 years later. Despite no significant change in axial length over this period (a difference of –0.04 mm), there was a 6.7% increase in FAZ area. This is due to a vessel in the upper right corner of the FAZ in (A, arrow) that is not visible in the follow-up image (B). Scale bar, 200 μm.

avascular zone morphometry (as well as errors in absolute foveal avascular zone measurements, as has been shown previously[13,14]). As retinal imaging becomes progressively more common in younger populations,[6,10–12] it is critical that accurate methods to account for eye growth (and therefore changes in lateral scale of retinal images) are developed and followed.[21]

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