Foveal Differentiation and Inner Retinal Displacement Are Arrested in Extremely Premature Infants

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Purpose. Children with a history of prematurity often have poorly developed foveae but when during development foveal differences arise. We hypothesize that the course of foveal development is altered from the time of preterm birth.

Methods. Eyes of 102 preterm infants undergoing retinopathy of prematurity screening examinations in the STudy of Eye imaging in Premature infantS (BabySTEPS) (NCT02887157) were serially imaged between 30 and 42 weeks postmenstrual age (PMA) using handheld optical coherence tomography systems. Total retinal thickness, inner retinal layer (IRL) thickness, and outer retinal layer (ORL) thickness were measured at the foveal center and parafovea. Foveal put depth, IRL thickness, and ORL thickness were compared between infants born at different gestational ages using mixed effects models.

Results. Foveal pit depth and IRL thickness were inversely related to gestational age; on average, the most premature infants had the thickest IRL and shallowest pits at all PMAs. Differences were evident by 30 weeks PMA and persisted through 42 weeks PMA. The foveal pits of the most premature infants did not progressively deepen, and the IRLs did not continue to thin with increasing chronological age.

Conclusions. Foveation in extremely preterm infants is arrested from the earliest observed ages and fails to progress through term equivalent age. The developmental displacement of the IRL from the foveal center into the parafovea does not occur normally after preterm birth. These observations suggest that foveal hypoplasia seen in children with history of prematurity is due to disturbances in foveal development that manifest within weeks of birth.

Keywords: fovea, prematurity, development, OCT, retina

The human fovea is characterized by unique specializations of both retinal inner and outer layers that support high-acuity central vision. In the outer retina, rods are excluded from the foveola, and foveal cones have an elongated morphology that facilitates high-density photoreceptor packing, producing focal thickening of the outer nuclear layer (ONL) under the foveal center. Meanwhile, the foveal inner retinal layers are centrifugally displaced from the foveal center to produce the characteristic concave cross-sectional profile of the foveal pit. The fovea is the only location at which the retina is not stratified into alternating cell body and synaptic layers, with the foveal center instead comprised exclusively of photoreceptors without overlying interneurons.[1]

Histologic studies have demonstrated that the location of the presumptive fovea is discernible by 22 weeks gestational age in humans because of increased density of retinal ganglion cells, concentration of cones, and absence of rods.[2,3] A foveal depression begins to emerge between 24 and 26 weeks, and the retinal nerve fiber layer, ganglion cell layer, inner plexiform layer, inner nuclear layer (INL), and outer plexiform layer are progressively extruded from the foveola until only the photoreceptor layers remain in the center of the foveal pit.[2–4] The fovea does not reach histologic maturity until one to two years of age, with the majority of inner retinal differentiation occurring before birth and outer retinal differentiation occurring after birth.[4–6] Thus, in cases of premature birth, parturition can precede and potentially affect important steps of foveal development.

Much of the fundamental knowledge about human foveal development has come from histology of postmortem samples, but small sample sizes, variability in tissue quality, and cross-sectional design have limited this approach. Optical coherence tomography (OCT) has provided an approach to image the human retina in vivo, and more recently the advent of handheld OCT devices has allowed the

noninvasive imaging of retinal cytoarchitecture in pediatric populations.[7–10] Using OCT, the developing macula can be followed noninvasively, and many of the key histologic features captured with high fidelity.[11]

OCT has allowed structural studies of foveal morphology in children and young adults with history of prematurity, a population in which histologic specimens are rare. Several groups have found that school-aged children and adults with history of prematurity display abnormally persistent retinal inner layers and thickening of the fovea.[12–19] Thus it seems that prematurity is a risk factor not only for vascular anomalies in retinopathy of prematurity (ROP) but also for lack of foveal differentiation.[20]

In this study, we used handheld OCT devices to obtain serial images of the macula in a large cohort of premature infants undergoing ROP screening.[21] The depth of the foveal pit was quantified, and inner versus outer retinal layer thickness measured in segmented images. We found that infants born at younger gestational ages had shallower foveal pits compared to later born infants throughout their nursery course, explained in large part by thicker inner retinal layers at the foveal center. Additionally, the fovea did not continue to mature with chronologic age in the infants with history of most extreme prematurity. Overall, this study suggests that the process of retinal remodeling that produces a mature fovea is disrupted by severity of prematurity, with differences emerging within weeks of birth.

Methods

The prospective observational BabySTEPS (STudy of Eye imaging in Premature infantS) was approved by the Duke University Health System Institutional Review Board and registered with clinicaltrials.gov (NCT02887157). The study adhered to the guidelines of Health Insurance Portability and Accountability Act and the tenets of the Declaration of Helsinki. The BabySTEPS enrollment, image capture, and reproducibility have been reported previously[21] and are summarized in brief here.

Participants

Premature infants were eligible for enrollment if they were undergoing ROP screening in the Duke University Hospital neonatal intensive care nursery, were clinically able to undergo examination and imaging, or did not have severe neurologic comorbidities (such as anencephaly), and the parent or legal guardian provided informed consent. Birth weight percentiles were calculated from their recorded birth weight in grams and gestational age at birth using the PediTools Growth Parameters online calculator according to the revised Fenton preterm growth charts.[21–23]

OCT Imaging

OCT images were acquired with either of the two investigational SSOCT systems using ultracompact handheld probes operating at 100 kHz (UC2) or 200 kHz (UC3) SSOCT system with one of two handheld probes[24,25] or, in a minority of cases, a commercially available handheld spectral domain (SD)-OCT system (Leica Envisu C2300; Leica Microsystems, Wetzlar, Germany). Imaging was performed on dilated eyes of unsedated infants at the time of their clinically indicated ROP examinations, or infrequently, nondilated that day. Images acquired in the intensive care nursery between 30 and 42 weeks postmenstrual age (PMA) were included in this analysis.

Image Analysis

A senior OCT image grader (KPW), masked to all patient information with the exception of the age at time of imaging session, manually selected the single B-scan that best captured the fovea and marked the foveal center for each set of images based on the presence of an inner retinal divot or at the thinnest point of the retinal nerve fiber layer. Semiautomatic segmentation of retinal layers in the central foveal frame across the entire scan was based on automatic segmentation using the proprietary infant-specific software, the Duke OCT Retinal Analysis Program Marking Code Baby version 1.2 or 2.0 (MATLAB; MathWorks, Natick, MA, USA) with manual correction by the same grader.[26] Total retinal thickness was measured from internal limiting membrane to retinal pigmented epithelium, inner retinal layers (IRL) were measured from internal limiting membrane to inner border of the INL, and the outer retinal layers (ORL) from the outer border of the INL to the inner border of the retinal pigmented epithelium. Parafoveal thicknesses were the average of thickness values at points a nominal distance of 1000 µm nasal and temporal to the foveal center. This distance was chosen because 1000 µm falls near the thickest region of the parafoveal retina, and data from this eccentricity were available in the preponderance of macular volumes. In images where either the nasal or temporal parafoveal location was not available due to decentered imaging volumes (84 of 1122 images), the available nasal or temporal location was used for parafoveal values.

Macular Edema and INL Thickening

Macular edema is common in premature infants and confounds morphologic assessment of foveal pit formation since macular edema often causes thickening of the retina at the foveal center. We used average INL thickness across the central 2000 µm of the foveal B-scan as a quantitative and unbiased index of macular edema. Macular edema was manually graded as present or absent by the same OCT grader (Supplementary Fig. S1A). Using receiver operating characteristic analysis, average INL thickness was found to excellently predict macular edema with an area-under-the-curve of 0.93 (Supplementary Fig. S1B). A cutoff value of 71.25 µm was found to maximize true positive rate and minimize false positive rate, with 81% sensitivity and 95% specificity. The 511 images from 127 eyes with average INL thicknesses over this value were excluded from analysis where indicated. Over all the images in this dataset there was little relationship between INL thickness and either IRL or ORL thickness (Supplementary Fig. S1C).

Foveal Pit Assessment

Foveal B-scans were segmented to measure total retinal thickness, IRL thickness, and ORL thickness as described. (Fig. 1). As an index of foveal pit differentiation, we defined the parafoveal/foveal ratio (P/F) as the average of the nasal and temporal parafoveal thickness divided by central foveal thickness. In this way, a larger P/F indicates a deeper foveal pit.

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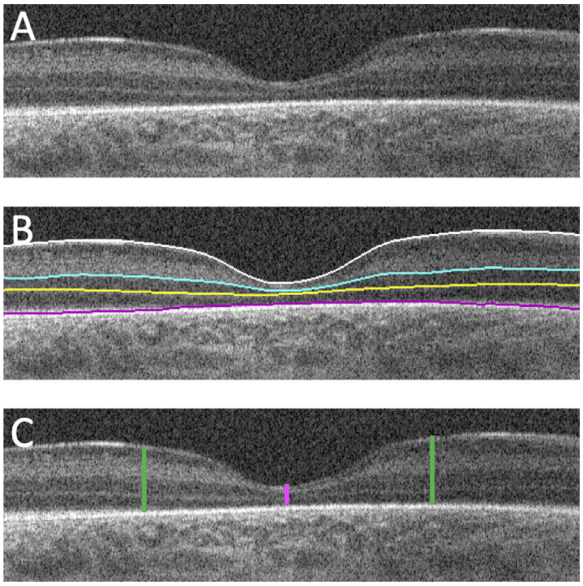

Figure 1. Retinal OCT segmentation and foveal pit measurement. **(A)** Example foveal OCT image from a preterm infant born at 32 weeks gestational age and imaged at 39 weeks postmenstrual age. **(B)** The same image as **A** with overlaid laminar segmentation. *White*: ILM; *cyan*: IPL – inner nuclear layer (INL) border; *yellow*: INL – outer plexiform layer (OPL) border; *purple*: inner border of retinal pigmented epithelium (RPE). Total retinal thickness was defined as ILM to RPE (*white* to *purple*), IRL from ILM to the inner border of the INL (*white* to *cyan*), and ORL from the outer border of the INL to the inner border of the RPE (*yellow* to *purple*). **(C)** Schematic representation of central foveal (*magenta*) and parafoveal (*green*) retinal locations. The P/F ratio, which represents foveal pit depth, is calculated as the average of the total neuroretinal thickness at 1000 µm nasal and temporal to the foveal center (*green lines*) divided by the thickness at the foveal center (*magenta line*).

Statistical Analysis and Data Visualization

We evaluated cross-sectional association between demographics of infants (e.g., birth weight, gestational age [GA] and OCT measures (e.g., maximum P/F ratio, minimum IRL thickness) using linear mixed effects models with random intercept for each infant to account for the inter-eye correlation. These analyses were performed without and with adjustment by the PMA at the latest image session. We performed longitudinal analysis for evaluating the effect of GA on OCT measures over time using linear mixed effects models. For longitudinal analysis, both the correlations over time within each eye and intereye correlation within each participant were accounted for by random intercepts for each eye nested within a random intercept for each participant. In longitudinal analyses, the predictors included GA, PMA and their two-way interaction, where both GA and PMA were modeled as continuous variables. In some cases where multiple imaging sessions were acquired for an eye within the same week of PMA, the first acquired imaging session was arbitrarily chosen for longitudinal analysis. GA and PMA were recorded as weeks completed for statistical analysis. GA was modelled as a continuous variable for *P* value calculation but was binned approximately into quartiles (GA ≤24, 25–27, 28–29, and ≥30 weeks) for data visualization and description. Summary statistics of OCT measures by GA groups from raw data were presented as mean (standard error) and displayed with lines representing linear fits of the raw data and shaded regions indicating 95% confidence intervals. Statistical analyses for the linear mixed effects models were performed in SAS v9.4 (SAS Institute Inc, Cary, NC, USA) and graphs produced in JMP Pro v15.0 (SAS Institute Inc).

Table 1. Participant Demographic Information and Imaging Characteristics

Characteristic	Data
Participants	102
Eyes	204
Female Participants	52 (51%)
Race	
Asian	5 (5%)
Black	47 (46%)
White	46 (45%)
Other/Multiple	4 (4%)
Ethnicity	
Hispanic or Latino	10 (10%)
Not Hispanic or Latino	92 (90%)
GA at Birth (weeks)	
Mean ± SD	27.6 ± 2.6
Range	23–34
≤24	17
25–27	27
28–29	37
≥30	21
Birthweight (g)	
Mean ± SD	981 ± 289
Range	420–1580
Maximum Stage ROP by Eye	
0	91 (45%)
1	31 (15%)
2	53 (26%)
3	28 (14%)
4	1 (0%)
ROP Treatment by Eye	
None	178 (87%)
Laser	6 (3%)
Intravitreal Bevacizumab + Laser	20 (10%)
Imaging Sessions	1122
Imaging Sessions per Eye	
Mean ± SD	5.5 ± 3.3
Range	1–13
PMA at Earliest Imaging (weeks)	
Mean ± SD	32.4 ± 1.8
Range	30–38
PMA at Latest Imaging (weeks)	
Mean ± SD	39.2 ± 2.7
Range	30–42
Images with INL Thickening	511 (46%)
Eyes with INL Thickening in Any Image	127 (62%)
Eyes with INL Thickening in All Images	27 (13%)

Data are reported as number (%) unless otherwise noted.

Results

A total of 1122 foveal B-scans from 204 eyes of 102 participants acquired between 30 and 42 weeks PMA were included in this study. The participant demographic information and imaging characteristics are summarized in Table 1. Five images (0.4%) were not of sufficient quality to segment the desired retinal layers and were excluded from all analysis. Five hundred eleven images (46%) had INL thickening above the predetermined maximum thickness and were excluded from some analyses where indicated, leaving 611

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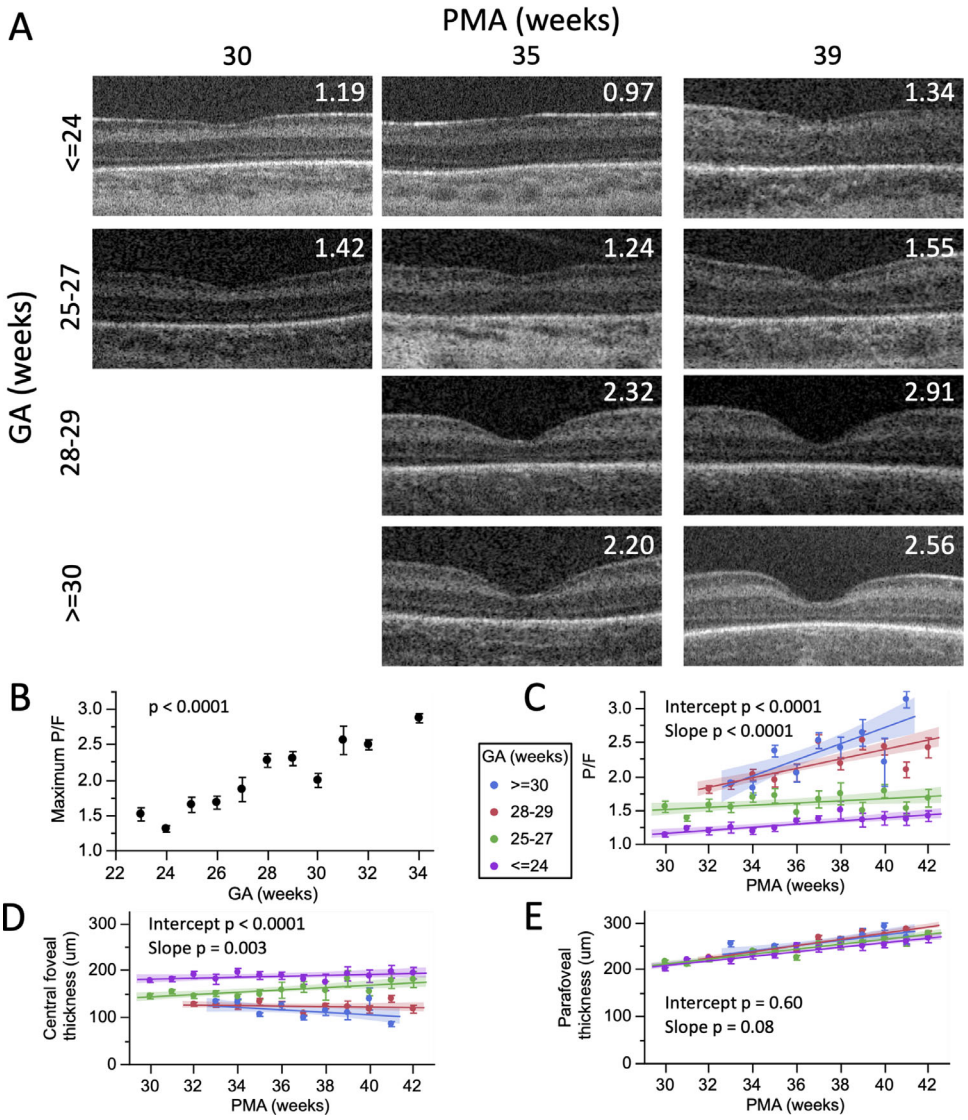

Figure 2. Extreme prematurity is associated with shallower foveal pits caused by thickening at the foveal center. **(A)** Example foveal OCT images from different infants born at a range of gestational ages (GA; columns) imaged across a range of PMA (*rows*). Inset numbers are the P/F ratio for the corresponding image. **(B)** Summary of the average maximum P/F observed per eye by GA. **(C)** Summary of average P/F across PMAs, excluding images with INL thickening. The study population was divided into GA quartiles for visualization. P/F is lower and increases less with PMA in more premature infants. **(D)** The neuroretina is thicker at the foveal center in more premature infants, and does not thin with increasing PMA. **(E)** Parafoveal neuroretinal thickness increases with PMA but is not affected by GA. Data displayed in this figure exclude images with INL thickening. *P* values represent the results of linear mixed model analysis with gestational age treated as a continuous variable (see Tables 2 and 3).

images from 177 eyes of 89 infants in the analyses without INL thickening. One hundred twenty-seven eyes (62%) displayed INL thickening at one or more imaging session, and 27 eyes (13%) had INL thickening at all imaging sessions (Table 1).

More Prematurely Born Infants Have Shallower Foveal Pits Throughout Early Postnatal Development

To determine whether prematurity might be related to foveal pit differentiation, we examined whether GA was related to foveal pit morphology. Foveal pits from extremely preterm infants appeared shallower and less well defined at both early and later PMAs (Fig. 2A). To quantify foveal pit depth, we analyzed the maximum P/F measured across all imaging sessions for each eye, excluding images with INL thickening (but not eyes with INL thickening in some sessions). We considered several patient factors as potential predictors of P/F: GA, birthweight (BW), birthweight percentile (BW %ile), maximum stage of ROP, ROP treatment, sex, race, and ethnicity. Because we expected P/F to increase with age and the PMAs at which images were available for each eye differed according to ROP exams and INL thickening, we also included PMA at the latest imaging session as a control factor. In univariate analyses, younger PMA at latest imaging session, younger GA, lower BW, lower BW %ile, more advanced ROP, and ROP requiring treatment were all associated with lower maximum P/F, whereas sex, race, and

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Table 2. Estimated Fixed Effects from Linear Mixed Models Predicting Maximum P/F Per Eye

	Univariate		Latest PMA Adjusted	
Factor	**Estimate (SE)**	***P* Value**	**Estimate (SE)**	***P* Value**
GA (per week)	0.15 (0.02)	<0.0001	0.14 (0.02)	<0.0001
BW (per 100 g)	0.09 (0.02)	<0.0001	0.09 (0.02)	<0.0001
BW %ile (per 10%)	−0.05 (0.02)	0.04	−0.04 (0.02)	0.08
ROP Maximum Stage		0.002		<0.0001
0	2.18 (0.08)		2.21 (0.07)	
1	2.08 (0.09)		2.09 (0.09)	
2	1.76 (0.09)		1.74 (0.08)	
3+	1.76 (0.10)		1.69 (0.10)	
ROP Treatment		0.01		0.0008
No	2.04 (0.07)		2.06 (0.06)	
Yes	1.58 (0.16)		1.48 (0.15)	
Sex		0.09		0.15
Male	1.86 (0.09)		1.89 (0.09)	
Female	2.09 (0.09)		2.07 (0.09)	
Race		0.09		0.10
Asian	2.41 (0.27)		2.41 (0.26)	
Black	2.06 (0.09)		2.06 (0.09)	
White	1.81 (0.10)		1.83 (0.09)	
Other/Multiple	2.19 (0.30)		2.08 (0.29)	
Ethnicity		0.99		0.77
Hispanic or Latino	1.98 (0.21)		1.92 (0.20)	
Not Hispanic or Latino	1.97 (0.07)		1.98 (0.07)	
Latest PMA (per week)	0.03 (0.01)	0.01		

The analysis reported in this table excluded images with INL thickening.

Table 3. Effect of GA on P/F and Central and Parafoveal Retinal Thickness Over Time

	P/F		Central Foveal Thickness (µm)		Parafoveal Thickness (µm)	
GA (Weeks)	**Estimate (SE)**	***P* Value**	**Estimate (SE)**	***P* Value**	**Estimate (SE)**	***P* Value**
Intercept (at 30 weeks PMA)						
Per week of GA	0.08 (0.02)	<0.0001	−7.7 (1.5)	<0.0001	0.45 (0.86)	0.60
24	1.15 (0.10)		181 (8.2)		206 (4.6)	
25–27	1.44 (0.08)		154 (6.6)		215 (3.7)	
28–29	1.68 (0.07)		131 (5.9)		215 (3.4)	
30	1.89 (0.14)		117 (11.3)		216 (7.1)	
Slope (per week PMA)						
Per week of GA	0.008 (0.030)	<0.0001	−0.29 (0.09)	0.003	0.12 (0.07)	0.08
24	0.026 (0.005)		1.22 (0.37)		5.89 (0.27)	
25–27	0.036 (0.004)		0.96 (0.35)		5.82 (0.25)	
28–29	0.071 (0.005)		−0.51 (0.37)		6.38 (0.27)	
30	0.060 (0.013)		−0.02 (1.09)		6.13 (0.79)	

P values reported from tests of linear trends. GA bins reported to correspond to quartiles displayed in figures. The analysis reported in this table excluded images with INL thickening.

ethnicity did not reach significance (Table 2). When latest PMA was included as covariate, GA, BW, and ROP remained statistically significant, whereas BW %ile was borderline (Table 2). Multivariate analysis exploring combinations of patient factors favored the simple model that included only GA adjusted for latest PMA, with the other factors not further improving the goodness-of-fit of the model. Overall, this analysis indicates that GA is the most important predictor of maximum foveal pit depth, with the most extremely preterm infants achieving P/F ratios approximately half as large as the least preterm infants by 42 weeks PMA (Fig. 2B).

To explore when the effect of prematurity on foveal pit depth manifests, we examined how foveal pit depth changed over time. When we looked at all images without INL thickening, we saw that average P/F increased with PMA (Figs. 2A, 2C), consistent with the known timing of foveal pit maturation during this developmental epoch. On the basis of the hypothesis regarding prematurity interfering with foveation and the results of our independent measures analysis (Table 2), we focused on how GA affected P/F longitudinally (Fig. 2C). Consistent with the results when analyzed by eye, retinas of younger born infants had lower P/F ratios that increased less with PMA (Table 3). Differences in foveal pit depth were already present by 30 weeks PMA when the earliest imaging sessions were conducted, reflected by the significantly different 30-week PMA intercept of the linear mixed effects models (Table 3). Altogether, these results indicate that more premature infants start with shallower foveal pits

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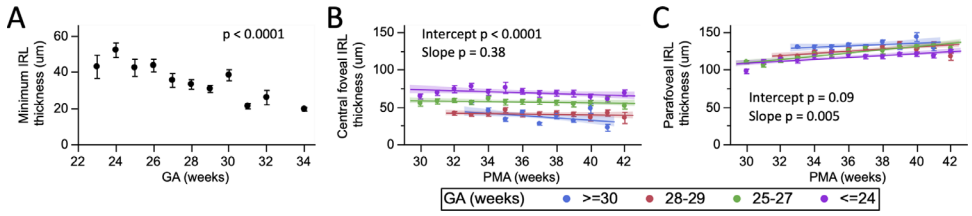

Figure 3. Extremely premature infants have persistently thicker inner retinal layers from soon after birth. **(A)** The minimum IRL thickness per eye depends on GA, with infants born at younger GAs maintaining thicker IRL. (**B**) Mean IRL thickness over PMA for all images regardless of INL thickening. IRL at the foveal center are thicker in infants born at earlier GAs, and there is little IRL thinning over time. (**C**) Conversely, parafoveal IRL are thinner in infants born at earlier GAs. Data displayed in this figure include all images regardless of INL thickening. *P* values represent the results of linear mixed model analysis with gestational age treated as a continuous variable (see Table 4).

Table 4. Effect of GA on IRL Thickness Over Time

	Central Foveal IRL Thickness (µm)		Parafoveal IRL Thickness (µm)	
GA (Weeks)	**Estimate (SE)**	***P* Value**	**Estimate (SE)**	***P* Value**
Intercept (at 30 weeks PMA)				
Per week of GA	−5.02 (0.69)	<0.0001	0.99 (0.58)	0.09
≤24	72.2 (3.6)		106 (3.2)	
25–27	60.5 (2.9)		111 (2.6)	
28–29	40.9 (2.9)		115 (2.4)	
≥30	39.9 (5.6)		123 (4.4)	
Slope (per week PMA)				
Per week of GA	0.06 (0.06)	0.38	0.13 (0.05)	0.005
≤24	−0.41 (0.22)		1.47 (0.15)	
25–27	−0.71 (0.21)		2.08 (0.15)	
28–29	−0.09 (0.29)		2.28 (0.21)	
≥30	0.44 (0.69)		1.35 (0.50)	

P values reported from tests of linear trends. GA bins reported to correspond to quartiles displayed in figures. The analysis reported in this table included all images regardless of INL thickening.

that show less progressive morphologic differentiation postnatally compared to those born later.

Prematurity is Associated with Thickening at the Foveal Center

We next sought to explore whether differences in foveal pit depth were driven by effects of prematurity on central foveal thickness, parafoveal thickness, or both. First, we looked at the correlation of P/F with both foveal and parafoveal thickness across all images. We found a strong, inverse relationship between central foveal thickness and P/F (Supplementary Fig. S2A), whereas there was little relationship between parafoveal thickness and P/F (Supplementary Fig. S2B), suggesting that differences in central foveal thickness might underly differences in the P/F ratio. Indeed, the central foveal was thicker in infants born at younger GAs and did not become thinner with advancing PMA (Fig. 2D, Table 3), whereas parafoveal thickness increased with PMA but was not affected by GA (Fig. 2E, Table 3).

Inner Retinal Layers of Extremely Premature Infants are Persistently Thicker at the Foveal Center and Thinner Parafoveally

Because premature retinas had shallower foveal pits and foveal center was the part of the retina most strongly related to P/F, we hypothesized that more premature infants would not display normal physiological thinning of the foveal IRL. Because we found that macular edema is selectively associated with INL thickening and had little effect on IRL thickness (Supplementary Fig. S1C), we included all images in the IRL analysis regardless of their INL thicknesses. Consistent with our hypothesis, older preterm infants achieved thinner IRL by 42 weeks PMA (Fig. 3A). Linear mixed effects model analysis demonstrated a significant relationship between minimum IRL thickness and GA (linear slope estimate −3.26 ± 0.59 µm per week GA, $P < 0.0001$). Additionally, the minimum foveal IRL thickness strongly correlated with the maximum P/F within individual eyes (Supplementary Fig. S2C).

We next evaluated the effect of GA on IRL thickness over time. At the foveal center, younger GA was significantly associated with thicker IRL at 30 weeks PMA (Fig. 3B, Table 4). There was little change in IRL thickness as infants aged, and the slope of the IRL thickness by PMA did not significantly vary based on GA. Consequently, younger GA predicted thicker central foveal IRL throughout the ages examined in this study, with the IRL of the most premature infants being on average 75% thicker than those born least prematurely (Fig. 3B. Table 4). Meanwhile, the parafoveal IRL grew thicker with advancing PMA but were not strongly related to GA (Fig. 3C); there was a trend toward thinner parafoveal IRL at 30 weeks PMA in lower GA infants, and a small but statistically significant effect of GA such that younger born infants showed less parafoveal IRL thickening with increasing PMA (Table 4). Results were similar when images with INL thickening were excluded from analysis to avoid potential effects of macular edema (Supplementary Fig. S3, Supplementary Table S1), except that the small progressive

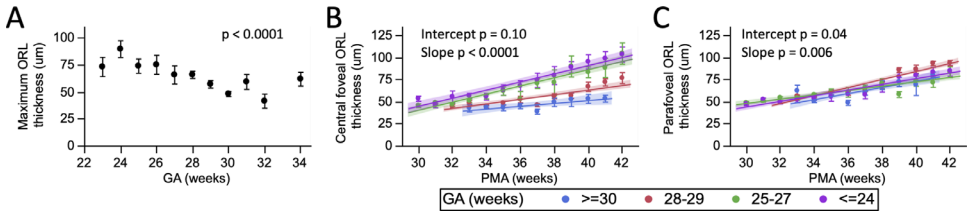

Figure 4. ORLs at the foveal center become thicker in more premature infants. **(A)** The maximum ORL thickness observed in each eye throughout the study depends on GA. Infants born at younger GAs attain thicker ORL. **(B)** Average ORL thickness at the foveal center across PMA for infants of different GAs. The ORL of more premature infants becomes progressively thicker with increasing PMA. **(C)** Parafoveal ORL thickness increases with PMA but is not affected by GA. Data displayed in this figure exclude images with INL thickening. *P* values represent the results of linear mixed model analysis with gestational age treated as a continuous variable (see Table 5).

Table 5. Effect of GA on ORL Thickness Over Time

	Central Foveal ORL Thickness (µm)		Parafoveal ORL Thickness (µm)	
GA (Weeks)	**Estimate (SE)**	***P* Value**	**Estimate (SE)**	***P* Value**
Intercept (at 30 weeks PMA)				
Per week of GA	−1.32 (0.80)	0.10	−1.14 (0.57)	0.04
≤24	47.8 (3.88)		46.0 (2.9)	
25–27	47.0 (3.15)		51.1 (2.3)	
28–29	39.0 (3.09)		42.3 (2.2)	
≥30	39.0 (7.22)		40.9 (4.9)	
Slope (per week PMA)				
Per week of GA	−0.41 (0.08)	<0.0001	0.14 (0.05)	0.006
≤24	4.59 (0.32)		3.39 (0.20)	
25–27	3.80 (0.30)		2.56 (0.19)	
28–29	2.40 (0.33)		4.12 (0.20)	
≥30	1.34 (0.92)		3.58 (0.58)	

P values reported from tests of linear trends. GA bins reported to correspond to quartiles displayed in figures. The analysis reported in this table excluded images with INL thickening.

thickening of parafoveal IRL with increasing PMA was no longer significant. Together these observations are consistent with the hypothesis that the displacement of inner retinal layers from the foveal center into the parafoveal region is perturbed by prematurity.

Extremely Premature Infants Have Abnormally Thickened Outer Retinal Layers at the Foveal Center

During normal development, specialization of foveal cones and thickening of the ONL occurs mostly postnatally.[11] We were, however, surprised to see that the ONL of many extremely premature infants developed a sharply peaked appearance at the foveal center when they reached older PMAs, whereas less preterm infants seemed to maintain a more uniform ONL thickness throughout the foveal region (Fig. 2A). Indeed, the maximum ORL thickness achieved by each eye was related to GA, with the mean ORL thickness of the earliest born infants approximately 70% greater than the latest born when images with INL thickening were excluded (Fig. 4A). Linear mixed effects model analysis demonstrated a significant relationship between maximum ORL thickness and GA (linear slope estimate −4.80 ± 0.78 µm per week GA, $P < 0.0001$). As with the IRL analysis, similar results were obtained for maximum ORL thickness whether or not images with INL thickening were excluded in the per eye analysis (Supplementary Fig. S4A).

When we examined ORL thickness longitudinally in images without INL thickening, we found that ORL thickness at the foveal center increased with PMA, but that the change with PMA was affected by GA (Fig. 4B). Central foveal ORL thickness was similar at 30 weeks PMA regardless of GA, but infants born at younger GAs showed more thickening with increasing PMA (Table 5). Parafoveal ORL thickness also increased with PMA, but the effect of GA was small (Fig. 4C, Table 5). When all images were included in the analysis regardless of INL thickening the pattern of results was overall similar, but the interaction of GA with PMA on central foveal ORL thickness was no longer significant (Supplementary Figs. S4B, S4C, Supplementary Table S2). It appears that macular edema could affect the ORL thickness of older GA infants at later PMAs and partially mask the effect of GA on ORL thickness. Overall, these observations show that eyes from infants born more prematurely have thicker ORL at the foveal center that become progressively more so with increasing PMA compared to older born infants.

Discussion

Our study used a large cohort of premature infants undergoing ROP screening to examine early foveal development using investigational handheld SSOCT devices to capture macular images. High-quality scans were reliably obtained in the earliest imaging sessions even in the most premature subjects. A range of foveal morphologies was observed, revealing that the degree of foveal differentiation in prema-

ture neonates was remarkably variable. Two related and crucial steps of foveation—formation of a foveal pit and displacement of the inner retinal layers from the central fovea—were more severely perturbed in infants with history of more extreme prematurity relative to later preterm infants. More preterm infants showed abnormal foveation from the earliest observed ages (30 weeks PMA), with both shallower foveal pits and thicker IRL at the foveal center. After premature birth, these two foveal parameters also failed to progress appreciably with age in the earliest born infants. These findings suggest that prematurity interferes with the normal developmental process of inner retinal foveation by preventing the displacement of the inner retinal layers from the foveal center. That the IRL thickness is affected by GA from as early as 30 weeks PMA and changes little postnatally raises the possibility that foveal differentiation is arrested at the time of parturition, though the latency between birth and first imaging and lack of PMA-matched in utero images prevents direct testing of this hypothesis.

Several previous studies have used OCT to describe immature foveal morphology and persistent inner retinal layers in premature infants.[27–30] In the largest previously published cohort of premature infants, researchers found progressive thickening of outer retina and thinning of the inner retinal layers at the central fovea with increasing PMA.[27] Taken altogether, our data are consistent with those observations. However, our study is the first to parametrically explore the relationship between prematurity and foveal maturation, revealing that the degree of prematurity exerts a profound effect on foveal development.

Our interpretation differs from a previously published smaller study of premature infants,[28] which concluded that, at least in eyes without macular edema or advanced ROP, foveal development after premature birth paralleled in utero development. Aside from differences in sample size, handling of macular edema could contribute to the different conclusions. We did not completely exclude eyes with macular edema from analysis; we found that although macular edema distorts total retinal thickness and blunts the foveal contour, laminar thickness measurements excluding the INL were relatively robust even in the presence of macular edema. Macular edema is common in preterm infants and is observed most frequently in infants born at younger GAs as they reach older PMAs. Excluding eyes that develop edema may select for a subpopulation that is not representative of preterm infants as a whole. In our data, the effect of GA on IRL persistence is observed whether or not eyes have macular edema. Thus IRL thickness can serve as a marker of foveal differentiation that can be followed even with fluctuating macular edema, and the inclusion of eyes with macular edema in our analysis where possible is a strength of this study.

Although the largest effects of prematurity were on the inner retina, we also unexpectedly observed progressive thickening of the ORL at the foveal center in extremely preterm infants. By term equivalent age, these eyes often featured a pyramidally peaked ONL centered under a shallow foveal depression. During normal development, foveal photoreceptors become elongated and densely packed over the first 1-2 years of postnatal life, resulting in thickening of the photoreceptor layers centrally on OCT.[31] The discrepant timing and minimal thickening in later preterm infants suggest that the early ONL thickening we found in extreme prematurity represents a process separate from the later physiologic thickening seen postnatally as a result of foveal photoreceptor specialization. Possibilities include primary photoreceptor abnormalities such as edema, abnormal cellular packing or migration, or other changes secondary to overlying inner retinal dysfunction.

Foveal pit morphology has previously been shown to vary by both race and sex in adults, with males having thicker retinas than females and Caucasians having shallower foveal pits than African Americans.[32] We did not observe significant effects of race or sex on P/F ratio in our data (Table 2). There are several possibilities, both biological and technical that could underlie this discrepancy. First, effects of prematurity on retinal development could prevent the manifestation of ethnicity or sex-dependent differences. Alternatively, different foveal measurements (i.e., P/F ratio versus foveal pit breadth or slope) might not be sensitive to anatomic diversity in different contexts. Finally, it is possible that ethnicity- and sex-related differences could emerge later in development as the retinal continues to remodel and mature.

Although the foveal pit is one of the most conspicuous features of the human macula, its relationship to visual function has been a topic of much debate.[33–35] As well as being seen in prematurity, a blunted or absent foveal pit with continuous inner retinal layers can be an idiopathic or associated with genetic conditions including albinism and achromatopsia. A grading system for foveal hypoplasia on OCT has been proposed, in which lower grades are defined by poorly defined foveal depressions and persistent retinal inner layers and higher grades by an additional lack of outer retinal thickening at the foveal center.[36] Foveal pit morphology and inner retinal layer displacement seem to be variably related to photoreceptor specialization, such that appropriate thickening of photoreceptor layers can still occur even in eyes without well-developed foveal pits.[37,38] Outer retinal specializations have been reported to correlate more strongly with visual acuity than central foveal thickness in albinism, and people with history of prematurity and flat foveal contours can have good visual acuity[17,19,36,39] Overall, the relationship between foveal morphology and visual acuity remains imperfectly understood and may vary by disease state.

Normal foveal development in term infants continues in the first years of life, marked most conspicuously by thickening of the foveal photoreceptor layers and continued displacement of the inner retinal layers from the foveal pit.[2,3,11,31,40,41] Numerous studies have demonstrated that children and young adults with history of prematurity have less differentiated foveae, including shallower foveal pits and preserved overlying retinal inner layers, compared to term-born individuals.[12–17,42] The results reported here suggest that the course of foveal maturation in premature infants diverges from normal perhaps as early as the time of birth, and previous studies suggest that these early differences have enduring and likely permanent consequences for the structure of the retina. Longer follow-up of children imaged as premature infants could shed light on whether IRL thickness and pit morphology remain static, and what implications these anatomic anomalies have on visual function.

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