Retinal Capillary Blood Flow in Diabetic and Nondiabetic Women during Pregnancy and Postpartum Period

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PURPOSE. To evaluate the response of retinal capillary circulation to pregnancy in women with diabetes and to correlate microcirculatory changes with progression of retinopathy during pregnancy.

METHODS. A prospective follow-up study of 32 pregnant women with insulin-dependent diabetes and 11 nondiabetic pregnant women. Perimacular capillary blood flow measured noninvasively by retinal flowmetry in the inferior perimacular retina. Eleven nondiabetic pregnant women served as diabetic control subjects.

RESULTS. In diabetic women, blood flows, measured by small-box analysis, were 233 ± 69 (mean ± SD) arbitrary units (AU) during the first trimester, 248 ± 55 AU during the third trimester, and 238 ± 46 AU 3 months postpartum, compared with 204 ± 32, 195 ± 22, and 196 ± 34 AU in nondiabetic pregnant women (P = 0.007 between groups). A difference of the same magnitude was evident between the two groups when the mean of the 50th (P = 0.032), 75th (P = 0.004), and 90th (P = 0.007) percentiles of the individual pixel flow values were used in point-wise analysis. In nondiabetic pregnant women, the small-box mean value was 201 ± 36, and the mean of the 75th percentile value in point-wise analysis was 316 ± 49. Blood flow was lower in nonpregnant than in pregnant diabetic women during the third trimester (P = 0.023 and P = 0.012, respectively).

CONCLUSIONS. Compared with nondiabetic pregnant women, retinal capillary blood flow was higher in diabetic women during pregnancy and after delivery. Together with the hormonal and metabolic changes occurring during pregnancy, hyperdynamic retinal capillary circulation may contribute to the progression of retinopathy in pregnant diabetic women.

DURING pregnancy, physiological changes occur in the cardiovascular, hormonal, metabolic, hematologic, and immunologic systems.¹ By some of these mechanisms, pregnancy causes deterioration in the background retinopathy in diabetic women, even when good metabolic control is achieved and retinopathy is minimal.² Three, however, although pregnancy is a major risk factor for the progression of diabetic retinopathy in the short term,¹³ no long-term effects have yet been found.⁴ Because the circulation is increased during pregnancy in general,¹ hyperdynamic retinal circulation in pregnant diabetic women would be expected to contribute to the progression of retinopathy. Previous studies on retinal blood flow in such women have reported both increased⁵,⁶ and decreased⁷ volumetric blood flow.

The human retinal microcirculation is difficult to access because of its dimensions and location. Invasive methods of measuring retinal capillary blood flow, such as those based on digital fluorescein angiography, cannot be used during pregnancy.⁸ Until now, the only means during pregnancy to evaluate retinal capillary blood flow noninvasively has been the psychophysical blue field entoptic simulation method.⁹ Recently, methods based on confocal scanning laser Doppler technology have made it possible to perform objective noninvasive measurements of retinal capillary blood flow.

METHODS

The study was performed with the approval of the local institutional review board, in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Pregnant Diabetic Women

From November 1998 to May 2001, 57 consecutive women with insulin-dependent diabetes were recruited for the study at the Department of Obstetrics and Gynecology, Helsinki University Central Hospital, as soon as pregnancy was verified (usually between 5 and 10 weeks of gestation). These women were then referred to the Department of Ophthalmology, where they were studied at the 12th to 14th weeks, 24th to 26th weeks, and 34th to 36th weeks of gestation, and at 3 months and 6 months postpartum.

Nine women were excluded either because of obstetric complications or coexisting eye disease: one because of spontaneous abortion, one for induced abortion due to a high glycosylated hemoglobin level (12%–13%), six for preterm delivery, and one for retinopathy. Thus, retinal flowmetry (Heidelberg Retinal Flowmetry [HRF], Heidelberg Engineering, Heidelberg, Germany) images were acquired from 48 diabetic women.

Of these 48 diabetic women, 15 were excluded from the analysis because of poor quality retinal flowmetry images, mainly due to motion artifacts and poor fixation. One patient was lost because of...
withdrawal from the study after delivery. Thus, final statistical analyses were performed on data from 32 diabetic women.

The mean age of the pregnant diabetic women was 30 ± 4.5 years. Of these 32 women, 6 (18.8%) were smokers and 3 (9.3%) had asthma, 5 (15.6%) allergy, 2 (6.3%) epilepsy, 1 (3.1%) celiac disease, (3.1%) ulcerative colitis, and 3 (9.4%) hypothyroid. Thirteen (40.6%) had no additional disease. Data from diabetic women with (α = 5) and without (α = 27) preeclampsia were analyzed together.

**Nondiabetic Pregnant Women**

Fifteen nondiabetic pregnant women in the same age-range (31.5 ± 3.0 years) attending the Department of Obstetrics and Gynecology for monitoring of normal pregnancy could be recruited to participate in the study. Four women were unable to attend all eye examinations because of obstetric complications: early fetal loss, preeclampsia, preterm uterine contractions, and preterm delivery. Thus, 11 nondiabetic subjects were examined during the first and third trimesters, and at 3 months after birth.

All nondiabetic subjects had corrected visual acuities of 6/6 or better, normal ocular examination results, and no history of eye disease. None of them smoked or used any systemic or topical medication that affects ocular blood flow.

**Nonpregnant Diabetic Control Subjects**

Eleven nonpregnant diabetic women participating in prepregnancy planning at the Department of Obstetrics and Gynecology were examined once, and formed a nondiabetic group of diabetic control subjects. The mean age of these subjects was 30.3 ± 3.4 years. All of them had corrected visual acuity of 6/6 or better. One had received focal laser treatment in the study eye. Retinal findings in the nondiabetic control subjects were the following: 3 (27.3%) subjects with retinopathy (RP) at level 10, 4 (36.3%) RP level 20, 3 (27.3%) RP level 55, and 1 (9.1%) RP level 45. Of these 11 women, no one smoked and 1 (9.1%) had asthma, 1 (9.1%) allergy, and 1 (9.1%) psoriasis.

**Ophthalmic Examination**

Each diabetic woman and nondiabetic woman underwent a complete ophthalmic examination, including measurement of visual acuity, measurement of intraocular pressure (IOP) with a Goldmann applanation tonometer, biomicroscopic examination by indirect ophthalmoscopy, and color fundus photography. In all participants, IOPs were below 21 mm Hg.

**Fundus Photography**

Fundus photography of both eyes was performed through dilated pupils (by the use of 2 drops of tropicamide, 5 mg/mL) by a trained operator with a retinal camera (TRC 50A; Topcon Corp., Tokyo, Japan; Elitechrome 100 film; Eastman Kodak, Rochester, NY). The severity of diabetic retinopathy was assessed by means of two 50° color slides, one centered at the macula and the other at the optic nerve head. Photographs taken during pregnancy were compared with those taken before pregnancy, if available. Ocular history, including laser photoocoagulation, was checked in hospital records.

Photographs were evaluated by a retinal specialist (II), who was blinded to all clinical information. Retinopathy was graded by a modification of the Early-Treatment Diabetic Retinopathy Study (ETDRS) grading system, with ETDRS standard pictures serving for grading of the severity of retinopathy. For each eye, the maximum grade of retinopathy lesions was determined as the overall severity level for that eye (RP level). Retinal findings were classified into the following groups: (1) no retinopathy (RP level 10), (2) very mild retinopathy (RP level 20), (3) mild retinopathy (RP level 55), (4) moderate retinopathy (RP level 43), (5) moderate retinopathy, more extensive intraretinal microvascular abnormalities (IRMA; RP level 47), (6) severe nonproliferative retinopathy (RP level 53), and (7) proliferative retinopathy (RP level >53). All the color fundus photographs from nondiabetic subjects were graded as RP level 10 in the blinded grading. In addition, the number of microaneurysms was counted in each fundus image.

**Measurement of Serum Glycosylated Hemoglobin Concentration**

Serum glycosylated hemoglobin (HbA1c) concentrations were measured by ion-exchange high-performance liquid chromatography (Diamat; Bio-Rad Laboratories, Hercules, CA). Three mean measurements of HbA1c were used in the study: the mean of all HbA1c measurements taken during the first, second, and third trimesters.

The blood glucose level in diabetic women was measured by a fingertip prick and a blood glucose meter (Glucometer Elite; Bayer Diagnostics, Fernwald, Germany) before acquisition of the retinal flowmetry image. Women with blood glucose less than 3.5 mM were given a light snack, and those with blood glucose more than 9.0 mM were given extra insulin before image acquisition were acquired. Blood glucose during flow measurements was generally between 6 and 9 mM.

**Retinal Blood Flow Measurements**

The retinal flowmeter is a confocal scanning laser Doppler system that maps blood flow within the fundus and produces blood flow readings in arbitrary units. The principles of this apparatus have been described in detail elsewhere. The scanned area was a rectangle (20° × 2.5°) composed of 64 horizontal lines of 256 points. Images, all centered below the fovea, were taken by a single investigator (SL). Focus and sensitivity values were set to produce appropriate brightness in the area to be investigated. The camera was positioned 2 cm from the corneal surface. During image acquisition, a fixation point was placed at a distance of 2.5 meters, and after bilateral pupillary dilatation with tropicamide, the subjects were asked to fixate that target. Three or more repeated images were obtained from each eye. In the follow-up visits, the focus setting used at the initial visit was used whenever possible.

**Analysis from the Instrument Default Small-Box and Point-wise Analysis**

The right eye was chosen for the analysis, except in six cases in which the left eye was chosen because the images from the right eye were of poor quality. Good, high-quality images suitable for retinal blood flow measurements were obtained from 32 diabetic women, 11 nondiabetic control subjects, and 11 nonpregnant diabetic control subjects.

Images were analyzed by placing a 10 × 10-pixel square, the small box, on an area of interest free of motion artifacts and major vessels. The mean flow in the 10 × 10-pixel square was recorded, and the mean of three such recordings in three separate locations on the perfusion map was calculated. The position of the three squares, together with the outline of major blood vessels, was drawn on a transparent overlay placed on the computer screen to allow the same areas to be assessed during follow-up. The squares were placed in the inferior vascularized macula approximately 750 µm from the center of the fovea. The squares were located in a nonparallel fashion on the perfusion map to avoid possible coincident motion artifacts in the follow-up images (Fig. 1). The same images were also assessed by point-wise analysis. This technique has been described in detail previously. A single pixel-sized cursor was swept across the same areas.
as were analyzed in the 10 x 10-pixel squares. One hundred pixels per area were recorded in three different locations corresponding to the areas investigated in the small-box analysis. The 300 flow values were entered into a log file. The log files were sorted by flow, and different percentiles (25th, 50th, 75th, and 90th) of the set of individual pixel flow values were counted. The percentage of pixels with a flow of zero was also calculated. The photodetection sensitivity (DC) from the analyzed pixels was measured. Pixels with DCs of less than 70 or more than 200 were excluded. The brightness of the image—that is, the sensitivity—was set each time.

**Subgroups**

For subgroup analysis, the flow values of the pregnant diabetic patients were compared between groups created according to the following criteria: (1) duration of diabetes 17 or more years or less than 17 years, (2) RP level 20 or more or less than 20 at third trimester, (3) change in microaneurysm count or RP level from baseline to the third trimester, (4) change in RP level from baseline to the third trimester, and (5) HbA1c level equal to, more than, or less than the mean in each trimester.

Two patients who showed significant progression of retinopathy during pregnancy underwent fluorescein angiography 1 to 3 months postpartum. Two patients underwent bilateral panphotocoagulation, and three patients received local laser treatment (one because of local neovascularization, one because of leaking microaneurysms, and one because of local vitreous traction and hemorrhage from an avulsed temporal vein).

**Statistical Analyses**

Statistical analyses were performed on computer (BMDP for Windows, ver. 7.0; BMDP Statistical Software, Los Angeles, CA). Analysis of variance and covariance with repeated measures were performed to study changes in macular capillary blood flow during pregnancy and after delivery and differences between pregnant diabetic and nondiabetic women. For the comparison of point-wise analysis results, multivariate ANOVA was performed. One-way ANOVA with Bonferroni adjustment was used to compare macular capillary blood flow values between pregnant diabetic women and nonpregnant diabetic control subjects. \( P < 0.05 \) was considered statistically significant.

**RESULTS**

Retinal capillary blood flow was higher in the diabetic women than in the nondiabetic women throughout pregnancy and in the postpartum period (multivariate ANOVA, \( P = 0.009 \)). In women with diabetes, retinal capillary blood flow tended to increase during pregnancy until the third trimester and to be lower 3 and 6 months postpartum. However, this trend was not statistically significant. The results were essentially the same with the small-box mean and the means of the 50th, 75th, and 90th percentiles of point-wise analyses (repeated measures ANOVA, Table 1, Fig. 2). Among the nondiabetic subjects, there were nonsignificant decreases in blood flow from the first to the third trimester and the 3-month postpartum levels.

The pregnant diabetic women were also divided into subgroups for study of the effects of duration of diabetes, severity of retinopathy, progression of retinopathy, and mean serum HbA1c concentration on retinal capillary blood flow. The mean \( \pm \) SD HbA1c level in pregnant diabetic women was 7.17% \( \pm \) 0.96% in the first, 6.44% \( \pm \) 0.89% in the second, and 6.74% \( \pm \) 1.18% in the third trimester. These subgroups were compared by using the small-box mean blood flow and the means of blood flows in the 25th, 50th, 75th, and 90th percentile point-wise analysis. No significant differences in flows categorized according to these criteria appeared during pregnancy or after delivery.
FIGURE 2. Mean ± SD values of the 75th percentile point-wise analysis of retinal capillary blood flow (in AU) in diabetic women (n = 32) and nondiabetic women (n = 11) during pregnancy and after delivery. P = 0.004 between groups, repeated measures ANOVA.

To study blood flow in the diabetic women with the most pronounced progression of retinopathy during pregnancy, we divided those with diabetes into two groups according to whether they underwent laser treatment during the follow-up. Table 2 shows the mean of the 75th percentile point-wise analysis blood flows in diabetic women who were laser-treated during follow-up, in non-laser-treated diabetic women, and in nondiabetic women. No difference appeared in the retinal capillary blood flow between treated and nontreated women with diabetes, but flows were lower in the nondiabetic than in the diabetic women. In Table 3, means of the 75th percentile of the individual-pixel point-wise analysis are reported separately in the two laser-treated diabetic women with the most severe progression of retinopathy during pregnancy and after delivery.

Of the 32 diabetic women, 17 (53.1%) had an RP level of 10 in the study eye at baseline. Six (18.8%) had an RP level of 20, 7 (21.9%) an RP level of 35, and 2 (6.3%) an RP level of 43. Two of the diabetic women had undergone panphotocoagulation at baseline. By the third trimester, 13 (40.6%) had an RP level of 10, 4 (12.5%) an RP level of 20, 10 (31.3%) an RP level of 35, 3 (9.3%) an RP level of 43, and 2 (6.3%) an RP level higher than 53. Significant progression of retinopathy was thus observed in only those two patients who showed development of bilateral neovascularization: one during the second and the other during the third trimester. These two patients underwent panphotocoagulation in the study eye during pregnancy. After panphotocoagulation, both patients showed a moderate decrease in retinal blood flow (Table 3). During pregnancy, 19 (59.4%) of the diabetic women had no progression in the level of retinopathy, 4 (12.5%) progressed one level, and 9 (28.1%) progressed more than one level. Because of the waxing and waning course of retinopathy—that is, increase in background retinopathy lesions (microaneurysms, cotton wool spots, hemorrhages) during pregnancy and decrease in these lesions after delivery, by 6 months postpartum, 22 (68.8%) diabetic women had the same level of RP as at baseline, and 10 (31.3%) had progressed. In addition, three of these patients received local laser treatment after the 3-month follow-up.

Blood flows measured in the nonpregnant diabetic women were also compared with those measured in the pregnant diabetic women during the third trimester. In the nonpregnant women, the small-box mean was 201 ± 36, and the means of the 50th, 75th, and 90th percentiles of the point-wise individual-pixel analysis were 150 ± 43, 316 ± 49, and 470 ± 70.

The small-box mean blood flow did not differ between nonpregnant and pregnant diabetic women during the first trimester (P = 0.58), but the level was lower in the nonpregnant diabetic women during the third trimester (P = 0.023) and 3 months after delivery (P = 0.05). For the mean in the 75th percentile of the point-wise analysis, the results between these two groups were essentially the same (P = 0.49, P = 0.012, P = 0.084). The small-box mean or the mean of the 75th percentile of the individual-point-wise analysis in nonpregnant diabetic women did not differ significantly from the blood flow levels in nondiabetic pregnant control subjects during the first and third trimester or 3 months postpartum (P = 0.99; one-way ANOVA with Bonferroni correction).

DISCUSSION

The main finding of the study was that retinal capillary blood flow is higher in women with insulin-dependent diabetes than in nondiabetic women during pregnancy and after delivery. In the patients with diabetes, mean retinal capillary blood flow tended to increase from the first to the third trimester and decrease after delivery, but these changes did not reach statistical significance. In contrast, no tendency towards an increase in mean retinal capillary blood flow was seen in the nondiabetic women. In addition, no difference existed in blood flow between nonpregnant diabetic control subjects and nondiabetic pregnant control subjects, whereas pregnant diabetic women had higher flow values than did nonpregnant diabetic control subjects.

The results obtained with the retinal flowmeter system show a relatively large intersession variation that may reduce its power to detect time-dependent changes. The magnitude of the increase caused by diabetic pregnancy compared with that caused by diabetes in general is somewhat unclear. Blood flows were higher in pregnant diabetic women during the third trimester than in a control group of nonpregnant diabetic women, but no significant difference was evident between nonpregnant diabetic women and nondiabetic pregnant women at any time point. These data suggest that the increase observed in retinal blood flow in diabetic women during pregnancy was at least in part connected with pregnancy, thus indicating an altered response of the retinal circulation to

<p>| Table 2. Individual Pixel Flow in the 75th Percentile by Point-wise Analysis in the Three Study Groups |
|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|---------------------|</p>
<table>
<thead>
<tr>
<th><strong>Patient Group</strong></th>
<th><strong>n</strong></th>
<th><strong>First Trimester</strong></th>
<th><strong>Third Trimester</strong></th>
<th><strong>3 Months Postpartum</strong></th>
<th><strong>P within Group</strong></th>
<th><strong>P vs. Control</strong></th>
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<tbody>
<tr>
<td>Laser-treated</td>
<td>5</td>
<td>341 ± 63</td>
<td>391 ± 59</td>
<td>395 ± 57</td>
<td>0.486</td>
<td>0.004</td>
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<tr>
<td>Not laser-treated</td>
<td>27</td>
<td>381 ± 124</td>
<td>393 ± 86</td>
<td>365 ± 80</td>
<td>0.340</td>
<td>0.008</td>
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<tr>
<td>Control</td>
<td>11</td>
<td>324 ± 57</td>
<td>301 ± 56</td>
<td>307 ± 57</td>
<td>0.423</td>
<td></td>
</tr>
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</table>

Data are expressed as the mean ± SD in AU. Of the laser-treated diabetic women, two received panphotocoagulation treatment, and three received local laser treatment between the second and third trimesters. Probabilities are based on repeated-measures ANOVA.
pregnancy in diabetes. Blood flows in diabetic women remained above the level in nondiabetic women at 3 months postpartum, and remained unchanged at 6 months postpartum, when the pregnancy-related cardiovascular circulatory changes should have been normalized. Our results are, however, somewhat inconclusive as to the timing of the normalization of retinal blood flow during the postpartum period.

Pregnancy provides a good model for the study of progression of retinopathy in diabetes, because retinal status often worsens during any defined period. To our knowledge, this is the first study to use direct objective measurement of capillary blood flow during pregnancy in diabetic and nondiabetic women. Previous studies involving total retinal blood flow, as measured from the main retinal vessels, have yielded conflicting results. Chen et al., measuring volumetric blood flow in the major retinal veins during pregnancy in diabetic women, found a connection between progression of retinopathy and increased volumetric total blood flow in a retinal quadrant. Although these results mostly likely changes in volumetric blood flow at the microcirculatory level, there was no direct measurement of blood flow velocity in retinal capillaries. Another study showed a decrease during pregnancy in volumetric retinal blood flow in diabetic women with no retinopathy \( (n = \text{4}) \) or with background retinopathy \( (n = \text{3}) \). It is difficult to relate these observations directly to local measurements of retinal capillary blood flow, because the distribution of changes in blood flow and the proportion of perfused capillaries among different retinal regions may vary. Data by Hellstedt et al., using the psychophysical blue-field entoptic simulation technique, suggest an increase in retinal capillary blood velocity during pregnancy in diabetic, but not in healthy, women.

Investigators in previous studies have also reported conflicting results concerning retinal blood flow in nonpregnant diabetic women. In a study using a retinal flowmeter, increased capillary blood flow was found in nonpregnant diabetic patients with background or preproliferative retinopathy, which is in agreement with our findings in pregnant diabetic women. Another study found total retinal blood flow measured by laser Doppler velocimetry to be elevated by 23.4% in diabetic subjects with background retinopathy and poor glycemic control, compared with values in control subjects. A mild increase in retinal volumetric blood flow was reported in diabetic patients with a duration of disease of less than 4 years and before the development of any clinically detectable retinopathy. In contrast to the results from Grunwald et al., Andren et al. reported a significant reduction in retinal capillary blood velocity measured from digitized fluorescein angiograms in diabetic patients in comparison with healthy subjects.

We measured blood flow in the perimacular region below the fovea, because measurements temporal to the fovea would have been technically difficult. In diabetic retinopathy, the earliest changes are known to occur in the perimacular region, most often in the area temporal to the fovea. Technically, it is easier to scan the peripapillary region of the retina than the macular area. Cuypers et al. showed that valid retinal flowmeter measurements are more likely to be obtained from the papilomacular region than the foveal area. In another study of diabetic patients, microaneurysms and acellular capillaries were more than twice as common in the superior than in the inferior retina. Chung et al. reported that in healthy people, baseline blood flow in the inferior temporal quadrant is significantly greater than in the superior temporal quadrant, suggesting that the inferior temporal retina has slightly greater capillary perfusion. Young diabetic women were a suitable study group for the retinal flowmeter method. Few of them had media opacities limiting retinal scanning, and most of them were capable of maintaining stable fixation during the 1.6-second data-acquisition period.

We used both the conventional 10 × 10-pixel square (small-box) and point-wise analysis to estimate retinal capillary blood flow. The reproducibility of retinal flowmeter measurements has been shown to be improved if point-wise analysis is used because the pixels with DCs of less than 70 or more than 200 can easily be excluded to improve measurement reliability. Data increased in underexposed images and decreased in overexposed images could mean less accurate results with the conventional 10 × 10-pixel small-box method, in which one DC represents the whole area. The results obtained in our series with both of these methods were similar, however.

Most diabetic women in our study had reasonably good glycemic control and only minimal or moderate retinopathy. Retinal capillary blood flow in patients with more drastic progression of retinopathy may differ. Today, however, improved obstetric care, improved glycemic control before and during pregnancy, and improved insulin therapy with new insulin analogues have reduced the number of diabetic patients who are at risk for development of severe forms of retinopathy because of pregnancy.

In summary, the retinal capillary blood flow was higher in diabetic women during pregnancy and after delivery than in nondiabetic pregnant women. Hyperdynamic retinal capillary circulation may contribute to the progression of diabetic retinopathy, or alternatively, our findings may reflect only an altered vascular system in diabetic women and the fact that pregnancy facilitates the progression of retinopathy by an independent, but parallel, causative pathway.

References


<table>
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<th>Patient</th>
<th>First Trimester</th>
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<th>Third Trimester</th>
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<th>6 Months Postpartum</th>
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Data are expressed as the mean flow in AU. Patient 1: Bilateral panphotocoagulation started during the 34th week of gestation. Neovascularization was still active 6 months postpartum, with bilateral macular edema. Visual acuity had decreased to 10/20 OD and 8/20 OS at 6 months postpartum. Patient 2: Bilateral panphotocoagulation started during the 29th week. Regression occurred by 6 months postpartum. Full visual acuity (20/20) in both eyes at 6 months postpartum.