The Foveal Avascular Region of Developing Human Retina

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Objective: To study the development of the perifoveal retinal vasculature.

Methods: We studied 7 retinas aged between 26 weeks' gestation and 1 week postnatal (41 weeks' gestation). Sections were imaged using high-resolution digital photography and blood vessel profiles identified at 200% to 300% magnification. Flat mounts were immunolabeled using antibodies to CD31 and factor VIII to identify blood vessels and antibodies to rhodopsin to identify the rod-free zone.

Results: The foveal region was identified by the absence of rod photoreceptors in the outer retina and/or presence of a shallow depression in the inner retina. The whole mount at 26 weeks' gestation showed a blood vessel–free region centered on the rod-free zone that was open along the horizontal meridian on the temporal side. At 37 weeks' gestation, the foveal avascular zone formed a complete circle. In sections, the foveal avascular zone was approximately 500 µm in diameter at 35 weeks' gestation and 300 to 350 µm at 40 weeks' gestation; in whole mounts, it was 150 to 170 µm in diameter at 37 and 41 weeks' gestation.

Conclusions: The foveal region is normally avascular during development, as in adult life. We found no evidence of foveal vascularization during development of the human retina.

Clinical Relevance: Instances of vascularization of the foveal region are not due to failed regression of a transient vasculature.


The adult human fovea has a unique structural organization. Its most obvious feature is a shallow pit in which the ganglion cell, inner plexiform, and inner nuclear layers are displaced peripherally, forming a thick rim or slope around the pit (Figure 1A). In the center of the fovea, or foveola, only the photoreceptor layer remains and this consists of tightly packed, elongated cones and Müller cell processes. Rods are excluded from the fovea, forming a central rod-free zone where cone density reaches more than 200 000/mm², the highest in the retina. All adult primate foveae contain a central avascular region known as the foveal avascular zone (FAZ) (Figure 1B), because blood vessels are found on the slope (Figure 1A) but not in the foveola. Studies from our laboratories have shown that the foveal pit and the peak cone density are a product of complex developmental processes that occur during the last half of gestation and the early postnatal years. Using a combination of finite element analysis and quantitative morphology, Springer and Hendrickson have developed a model of primate foveal development in which they suggest that the presence of an FAZ is a critical requirement for formation of the foveal pit. Furthermore, several studies using optical coherence tomography now indicate that absence of the FAZ is associated with absence of a foveal depression and reduced uncorrectable visual acuity.

Earlier investigations on the development of macular capillaries in macaques indicated the presence of a fully vascularized macula in fetal retinas from 125 days until about 3 weeks postnatal by India ink injection, though conflicting results were obtained from whole mounts stained using periodic acid–Schiff reagent, which showed the foveal region to be avascular throughout development. More recent studies of macaque and marmoset retinas using vascular-specific markers show that the FAZ is defined by the time the pit begins to form. In both species, blood vessels grow from the optic disc toward the foveal region, but blood vessels growing along the horizontal meridian grow slowly, while those superior and inferior skirt around the incipient fovea. Studies of monkeys show that these blood vessels meet along the horizontal meridian to form the FAZ first on the nasal side then slightly later on the temporal side of the incipient fovea. They also show that neither the developing blood ves-
The incipient fovea at 22 weeks’ gestation and the presence of vascular elements in temporal-superior and temporal-inferior retina are approaching, but have not yet reached, the foveal avascular zone (FAZ). Both the ganglion cell layer (GCL) and inner nuclear layer (INL) are represented by an indentation, appearing first in the ganglion cell layer and later in the inner nuclear layer.5,18,20,21

It has not been firmly established that an identical process takes place in development of the human macula. Previous studies17 have shown that retinal blood vessels first can be identified at the optic disc of human retina at 14 weeks of gestation and that these form 4 lobes (each representing the territory of a quadrantic artery) that grow relatively quickly into nasal, temporal-superior, and temporal-inferior retina. However, development of the retinal vascular system in temporal-superior and temporal-inferior retina is approaching, but have not yet reached, the incipient fovea at 22 weeks’ gestation and the presence of an FAZ at around 25 weeks’ gestation has been reported.24 Thus, there is no evidence for vascularization of the foveal region of human retina before 25 weeks’ gestation.

Despite this, another study using fluorescence angiography in human retina has reported “that the FAZ in developing humans is initially densely vascularized.”25 These authors propose that the FAZ develops by “apoptotic remodeling” after 36 weeks’ gestation.25 These conclusions are not consistent with any findings from other primate models. In this article, we present morphological evidence that the human FAZ and foveal depression remain avascular throughout development, similar to what has already been described for macaque and marmoset monkeys.

METHODS

Fetal human eyes were obtained, with institutional review board approval from the University of Washington, at 26 (n=2), 37, 40 (n=2), and 41 weeks’ gestation from infants who died after birth of causes that should not have affected retinal development. One fetus was born at 34 weeks’ gestation and survived 11 days to the equivalent of 35 to 36 weeks’ gestation. Eyes were fixed in 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) for 1 to 7 days. One eye at 26 weeks’ gestation and 1 at 40 weeks’ gestation were embedded in paraffin, and the eyes at 35 and 41 weeks’ gestation were embedded in glycol methacrylate. These eyes were serially sectioned through the central retina and stained with either hematoxylin and eosin or azure II and methylene blue. Both the ganglion cell layer and inner nuclear layer (ONL) are embedded in glycol methacrylate and stained with azure II and methylene blue.

Figure 1. The adult human fovea. A, Section through the adult fovea embedded in glycol methacrylate and stained with azure II and methylene blue. Both the ganglion cell layer (GCL) and inner nuclear layer (INL) are absent from the foveola. Retinal blood vessels are present on the upper foveal slope (arrows) but not on the lower slope or in the foveola. B, Adult retina treated histochemically to show nicotinamide adenine dinucleotide phosphate-diaphorase activity in nitric oxide synthase–containing blood vessels and neurons. The most central retinal blood vessels in this flat mount are indicated with arrows. FAZ indicates foveal avascular zone; fH, fibers of Henle; IS, inner segments of photoreceptors; ONL, outer nuclear layer; OS, outer segments of photoreceptors; RPE, retinal epithelium layer.
rod-free zone). The labeling of blood vessels in the inner retina and in rods in the outer retina could be distinguished owing to the different focal levels in the confocal microscope. Low-power images were collected using a 10 × objective lens at the level of the photoreceptor layer; then at the same location, a z-stack through the inner blood vessel layer was acquired. Images of the rod photoreceptors were pasted into the green channel and images of the corresponding blood vessel were pasted into the red channel of an Adobe Photoshop image. All images were prepared for publication using Adobe Photoshop CS2.

In serial sections, the incipient fovea was identified by the presence of a wide region in the outer nuclear layer that contained a single layer of cones and no rods, the rod-free zone. Consistent with previous reports, at 26 weeks' gestation, the rod-free zone (RFZ) extends from the arrowhead in the outer nuclear layer (ONL) to beyond the field of view to the right. The approximate center of the RFZ—the incipient fovea—is shown to the right of the image. Blood vessels (arrows) are present on the nasal side of the fovea at the ganglion cell layer (GCL)/inner plexiform layer border. No blood vessels are present in the RFZ. B, This fetus survived 11 days after birth at 35 weeks' gestation. The most central rods are indicated with arrowheads. C, The retina of a fetus at 40 weeks' gestation. Blood vessels closest to the developing fovea (arrows) define an FAZ approximately 320 µm in diameter. The most central blood vessels are indicated with arrowheads. D, Retinal section from a donor who died 1 week after birth (41 weeks' gestation), showing a relatively immature fovea forming within an FAZ (arrows) approximately 350 µm in diameter. The most central rods are indicated with arrowheads. A, B, and D stained with azure II and methylene blue; C stained with hematoxylin and eosin.

Figure 2. Photomontages showing the relationships of rods and blood vessel profiles (outlined) to the developing fovea. A, Retina of a fetus at 26 weeks' gestation. The rod-free zone (RFZ) extends from the arrowhead in the outer nuclear layer (ONL) to beyond the field of view to the right. The approximate center of the RFZ—the incipient fovea—is shown to the right of the image. Blood vessels (arrows) are present on the nasal side of the fovea at the ganglion cell layer (GCL)/inner plexiform layer border. No blood vessels are present in the RFZ. B, This fetus survived 11 days after birth at 35 weeks' gestation. The macula shows evidence of edema and some anoxic damage, but the blood vessel morphology is clear. Blood vessels closest to the developing fovea (arrows) define a foveal avascular zone (FAZ) 480 to 500 µm in diameter. The most central rods are indicated with arrowheads. C, The retina of a fetus at 40 weeks' gestation. Blood vessels closest to the developing fovea (arrows) define an FAZ approximately 320 µm in diameter. The most central rods are indicated with arrowheads. D, Retinal section from a donor who died 1 week after birth (41 weeks' gestation), showing a relatively immature fovea forming within an FAZ (arrows) approximately 350 µm in diameter. The most central rods are indicated with arrowheads. A, B, and D stained with azure II and methylene blue; C stained with hematoxylin and eosin.

Figure 3. A, Whole mount of a retina at 26 weeks' gestation (fellow to that in Figure 2A). Rods (green) are absent from the foveal region, which is surrounded by CD31/factor VIII reactive blood vessels (red) defining the foveal avascular zone (FAZ). On the nasal side, the ring is complete, but it has not closed along the horizontal meridian (Hz) on the temporal side. Single blood vessel processes (arrow) extend toward the foveal center. B, Immunolabeled human retinal whole mount of a retina at 37 weeks' gestation showing a small but complete FAZ. The innermost border comprises blood vessel loops with only a few single processes (arrow). C, Immunolabeled human retinal whole mount of a retina at 40 weeks' gestation showing an FAZ similar to that at 37 weeks' gestation but with a more complex surrounding blood vessel meshwork. The FAZ in B and C are both centered on the rod-free zone.

RESULTS

TWENTY-SIX WEEKS’ GESTATION

Paraffin sections of the fovea at 26 weeks' gestation showed blood vessels growing toward the fovea on its nasal side at the inner plexiform layer/ganglion cell layer border (Figure 2A). The most central blood vessels on the nasal side reached almost as far as the edge of the rod-free zone (Figure 2A), about 1000 µm from its center. In the whole mount of the eye at 26 weeks' gestation (Figure 3A), a meshwork of CD31+/factor VIII+ blood vessels surrounds the nasal side of the rod-free zone, overlapping the most central rods. On the temporal side, blood vessels were present superior and inferior to the horizontal meridian separated by a gap of 250 to 400 µm. The capillaries bordering the rod-free zone had many single processes extending radially toward the center of the incipient fovea (Figure 3A). The meshwork around the fovea was highly irregular, resembling immature retinal capillaries.

THIRTY-FIVE TO 37 WEEKS’ GESTATION

Sections through the developing fovea at 35 weeks' gestation showed loss of photoreceptors and inner retinal neurons, suggesting anoxic episodes during the survival period of this fetus (Figure 2B). Blood vessels were absent from the shallow pit area but were present on the surrounding foveal slope. On the foveal rim, a blood ves-
sellarplexus had formed in inner retina and crossed the inner
plexiform layer to form the deep plexus in the inner
nuclear layer. Near the pit, blood vessels were mainly in
the inner plexus where the most central blood vessel pro-
file defined an FAZ approximately 500 μm in diameter.
In the immunolabeled whole mount of the 37-weeks’ ges-
tation retina (Figure 3B), the capillary meshwork sur-
rounded the developing fovea and the (previously ra-
dial) vessels formed loops, defining an FAZ approximately
150 to 170 μm in diameter. The FAZ was coincident with
the rod-free zone. Very few single processes extended to-
ward the foveal center at this age, suggesting that radial
growth into the developing fovea was completed.

FORTY TO 41 WEEKS’ GESTATION

The paraffin sections through the fovea at 40 weeks’ ges-
tation (Figure 2C) showed a well-developed pit that ex-
cavated the ganglion cell, inner plexiform, and inner nuclear
layers, so that only 1 to 2 neurons remained in these lay-
ers. The most central blood vessels were located at the in-
ner plexiform layer/inner nuclear layer border. Blood ves-
sels were absent from the pit center, defining an FAZ approxi-
ately 300 to 350 μm in diameter. Blood vessels were
much more numerous on the foveal slope com-
pared with the specimen at 35 weeks’ gestation, and just
outside the fovea, blood vessels had grown throughout the
inner nuclear layer as far as the outer nuclear layer/outer
plexiform layer border. In the immunolabeled whole mount
at birth (Figure 3C), the FAZ was of a similar diameter to the
retina at 37 weeks’ gestation (150-170 μm), with smooth
blood vessel loops and virtually no single radial pro-
cesses extending into the central fovea. In general, the
meshwork around the neonatal FAZ was more dense than
at 37 weeks’ gestation, suggesting that blood vessel growth
continues in the inner retinal layers surrounding the fo-
vea late in gestation. In the sections at 41 weeks’ gesta-
tion (Figure 2D), the foveal pit was less well developed,
though the blood vessel distribution was similar to that
at 40 weeks’ gestation, showing a fovea-centered FAZ ap-
proximately 340 μm in diameter.

Both the immunolabeled whole mounts and stained sec-
tions used in this study show that the foveal region of
the human retina remains free of blood vessels between
26 weeks’ gestation and 1 week postnatal. Viewed in the
context of previous studies that examined specimens
younger than 26 weeks of gestation,13,19,22-24 the evidence
now indicates that the foveal region of the human retina remains avascular throughout development. While
measurements of the diameter of the FAZ were consist-
ently greater in sections compared with whole mounts,
both sets of data show a progressive decrease in the size
of the FAZ during the second half of gestation. Our pre-
sent findings on human FAZ development are consistent
with previous investigations of retinal vascular develop-
ment in macaque and marmoset retinas.5,16-19,26

In human retina, blood vessels begin to form at the op-
tic disc at around 14 weeks’ gestation, and expression of
vascular endothelial growth factor messenger RNA can be
detected at this age on both nasal and temporal sides of the
disc (J.M.P., unpublished data, 2002-2003). Blood ves-
sel formation occurs relatively slowly along the temporal
horizontal meridian. At 22 weeks’ gestation, blood ves-
sels in temporal retinas still form prominent lobes that ex-
tend several millimeters into superior and inferior retina
but reach only a millimeter or so along the horizontal me-
ridian toward the foveal region.22 At this age, central blood
vessels are still 2 mm from the rod-free zone center.22,23
In humans and monkeys, blood vessels throughout the pe-
ripheral retina advance along the ganglion cell layer/nerve fiber layer interface. By contrast, blood vessels grow-
ing toward the developing fovea cross the ganglion cell layer
and advance at the ganglion cell layer/inner plexiform layer
border,5,10,18 reaching the nasal margin of the incipient
foveal pit by 25 to 26 weeks’ gestation in humans. In this
study, we show that on the nasal side of the developing
fovea at 26 weeks’ gestation, blood vessels form a com-
plex meshwork with sprouts extending into the foveal re-
gion, while on the temporal side, blood vessels are still sepa-
rated into superior and inferior lobes along the horizontal
meridian. By 37 weeks’ gestation, these lobes have fused,
and the early fovea is encircled by fine blood vessels. The
newborn FAZ is similar but has a more complex mesh-
work and fewer single processes, suggesting continued
blood vessel formation outside the fovea, at least until birth.
We found no evidence for overgrowth of the foveal cen-
ter by blood vessels in any of the specimens in this study,
including the 35 weeks’ gestation specimen that sur-
vived after premature birth and showed some evidence of
retinal anoxic stress. Between 37 and 40 weeks’ ges-
tation, we found that the FAZ diameter remained at 150 to
170 μm, showing no evidence of the enlargement pro-
posed previously to take place during the last weeks of fe-
tal development.24 Rather, our data suggest that remodel-
ing of the FAZ to adult dimensions (500-700 μm diameter25,28) occurs at least up to around 15 months af-
after birth, as the width and depth of the foveal pit change
to become more adultlike (compare Figure 1A and
Figure 2C and D).2,6,20 Similar data indicating that the FAZ
of marmoset retina is remodeled as the foveal pit matures
have also been reported recently.18

A major point of the retrospective study by Mintz-
Hittner et al25 was that humans born before 30 weeks’ ges-
tation will have a small or absent FAZ, while those born
after 36 weeks’ gestation will have a normal-sized FAZ.
These authors then conclude that the human FAZ forms
by a blood vessel overgrowth of the incipient fovea fol-
lowed by a clearing of this region owing to cell death be-
tween 30 and 36 weeks’ gestation. Several points should
be stressed about those conclusions. The first is that the
FAZ was studied in 1- to 17-year-old eyes, not fetal eyes,
so they provide no direct evidence for this postulated over-
growth during normal development. By contrast, in the
present study, we show a clear FAZ at 26, 35, 37, 40, and
41 weeks’ gestation, with no evidence of overgrowth. The
second is that the FAZ was visualized in vivo by fluo-
rescence angiography and no retinas were examined histo-
logically. Although the angiograms are of high quality, they
do not provide the fine resolution given by immunola-
beled whole mounts. Finally, no evidence is presented for
the postulated apoptotic pruning. Unpublished studies from our laboratory of FAZ development in macaque retinas found an extremely low level of apoptosis within perifoveal blood vessels and astrocytes at ages corresponding to human retinas at 30 to 36 weeks' gestation (Trent Sandercoc, PhD, MBBS, and J.M.P., unpublished data, 2002). Likewise, in macaque prenatal and postnatal foveae, Dister et al.29 detected only low levels of apoptosis in astrocytes and did not report significant cell death in adjacent blood vessels. Furthermore, in an extensive morphological study of human retinal blood vessel development, Hughes et al.26 describe vascular remodeling as a mechanism but report no evidence for extensive cell death. In our study, all infants, except one, died shortly after birth and, to the best of our knowledge, were at the correct gestational age and weight. Therefore, while the present population represents normal development, many of those in the previous study25 appear to show the effects of severe prematurity on foveal vascular development.

Considerable evidence from hypoplastic human retinas support the negative consequences of failure of the FAZ to form normally. If blood vessels invade the future foveal pit region, a shallow, incomplete pit and decreased visual acuity result.3,11 In addition, a recent morphological study of 2 neonatal human anencephalic retinas28 found overgrowth of the foveal region by blood vessels in 1 retina that was associated with failure of pit formation and formation of an FAZ with a shallow foveal pit in the second neonatal retina. Both neonatal anencephalic retinas lacked any central ganglion cells, but differed markedly in FAZ and pit formation, suggesting that ganglion cells are not directly involved in either process.

The findings from anencephalic retinas28 and hypoplastic foveal syndromes8-13 are consistent with the Springer model of foveal development, which predicts that if an FAZ is lacking, no pit will form.20,21 The model suggests that the more-elastic retina within the FAZ is initially indented by intraocular pressure, forming a narrow deep pit. Around birth in humans, accelerated retinal growth stretches the pit, making it wider and more shallow, at the same time promoting the later phase of cone packing to increase peak cone density. If the typical sequence of human development was for blood vessels to overgrow the future fovea, the model predicts that a fovea would not form and that cone density would be preserved at approximately the same density as established by 25 to 30 weeks' gestation, around 30,000 cones/mm2.3,4 Therefore, until significant morphological evidence is presented to the contrary, we assert that development of the human foveal depression involves the formation of a small FAZ by late gestation that actively enlarges as the pit remodels in the postnatal period.

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