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Retinal microvasculature measurements in full-term newborn infants

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Abstract .**Objective:** Currently, there are no published data on retinal microvasculature size in human infants born at term. The purpose of this study was to determine the normal retinal microvasculature measurements in human infants born at term with normal birth weight and to compare these results with measurements in children and adults. **Methods:** Retinal arteriole and venule measurements were obtained in a cohort of 20 full-term infants. Digital retinal images were obtained from both eyes after pupillary dilation using a digital retinal camera. Measurements of vessel diameter were then obtained using semi-automated software. **Results:** Twenty infants (9 female infants and 11 male infants) were analyzed. The retinal arteriole diameter was 66.8–123.0 μm (mean, 85.5 (14.3) μm), and the venule diameter was 102.0–167.8 μm (mean, 130.0 (16.0) μm). There were no differences in the arterial or venule diameters between the male and female infants (83.2 (12.2) vs. 88.3 (16.9); $P = 0.4372$; 124.3 (16.0) vs. 137.0 (18.0); $P = 0.08$). The arteriovenous ratio was found to be 0.66 (95% CI 0.62–0.71). The coefficient of correlation between the retinal arterioles and venules was 0.56. The retinal arteriole and venule diameters increase as a person matures. The arteriovenous ratio also increases with age. **Conclusion:** In newborn infants, retinal venules are significantly larger than retinal arterioles. The arteriovenous ratio is smaller in neonates compared to adults indicating the retinal arteriole diameter increases at a different pace compared to retinal venule. Sex does not influence the retinal microvasculature size in infants. The presence of spontaneous retinal hemorrhage and the inability to account for refractive errors were the main limitations of this study.

Keywords: retinal arteriole, retinal venule, low birth weight, middle cerebral artery, intrauterine growth restriction

List of Abbreviations: LBW, low birth weight, ROP, retinopathy of prematurity

NBW, normal birth weight, AVR, arteriovenous ratio

Introduction

Low-birth-weight (LBW) (birth weight < 2,500 g) is now well-recognized as a risk factor for the development of vascular-related diseases in later life (Barker, 2004; Barker, 2006a; Barker, 2006b; Barker et al., 2005; Barker et al., 1989). LBW infants are a heterogeneous group, comprising newborns who are premature (< 37 completed weeks of gestation), growth-restricted (weight below the 10th percentile for their gestational age) , or a combination of both. The exact mechanism of this phenomenon remains to be fully understood, but there is increasing evidence to suggest that microcirculatory pathologies form the mechanistic link between in utero insult and the adult manifestation of illness (Liew et al., 2008a; Martin et al., 2000; Mimoun et al., 2009; Sasongko et al., 2010; Struijker-Boudier et al., 2007). The challenge has been to investigate microcirculatory changes in vivo. The human retina provides a unique opportunity for in vivo investigation of microcirculation, and changes in the retinal vessels have been identified in some individuals who had LBW as infants and later developed cardiovascular and/or renal disease (Chapman et al., 1997; Hellstrom et al., 2004; Liew et al., 2008a; McGeechan et al., 2009; Sun et al., 2009). The ability of retinal imaging technology to assess and measure the retinal microvasculature makes this an important assessment tool (Chapman et al., 2001; Liew et al., 2008b; Wong et al., 2004a).

The retinal microvasculature of premature infants is routinely assessed to detect and treat retinopathy of prematurity (ROP) (Section on Ophthalmology et al., 2006). However, there have been no published studies regarding the use of retinal imaging technology to assess the retinal microvasculature of well full-term infants. There are also no published data concerning normal

baseline measurements of retinal microvasculature size in full-term infants. **The objective of this study was to determine measurements of retinal microvasculature in human infants born at term with normal birth weight (NBW) (weighing 2,500-4,499 g).** We then compared these measurements with those from children and adults.

Materials and Methods

This study was performed in the Department of Neonatology, The Townsville Hospital, Queensland, Australia. The Department of Neonatology is a tertiary perinatal center responsible for more than 10,000 births each year. The study commenced in August 2010, and the data presented in this report are based on patients recruited until June 2011. This study was approved by the Townsville Health District Human Research Ethics Committee, was conducted in compliance with good clinical practice guidelines, institutional review board regulations, and written consent from parents, and was in accordance with the tenets of the Declaration of Helsinki. **Babies who needed respiratory support, surgery, infants of gestational diabetes mothers, were excluded, as were those with syndromes, prematurity, LBW or chromosomal abnormalities.** All assessments were performed within the first 7 days of life. **Only babies who were born at term (37 weeks of gestation completed) with NBW were included in this study.** After pupillary dilation, digital images of both retinas were obtained using a digital retinal camera (RetCam, Massie Laboratories, Dublin, CA, USA). Measurements of the diameters of the retinal vessels were then obtained using a predetermined protocol that first involved the identification of retinal vessels located 0.5-1 disc diameter from the margin of the optic disc (Figure 1). We provided pain relief with oral sucrose and local anaesthetic agents for the eye examination. Pupillary dilation was carried out using cyclopentolate and phenylephrine

ophthalmic drops. **The procedure was carried out by two persons – a neonatologist and a neonatal nurse. The most reliable images were obtained when the infant remained calm and has adequate papillary dilatation. The focus button on the control panel was used to obtain the clearest image. Still retinal images with optic disc in the centre were taken from both retinas .The sharpest image was then chosen for vessel measurement.**

Measurements of vessel diameter were then obtained using semi-automated software (Vesselmap, IMEDOS GmbH, Jena, Germany) (Grunwald et al., 2009; Johnson et al., 2007). The vessel diameter was computed as the distance between the walls within the vessel. The caliber of directly viewed vessels was determined by the size of the red cell column, because the vessel walls and peripheral plasma layer are nearly transparent (Archer et al., 2010). Vessels were measured in each eye, and the largest venule and arteriole of each patient was determined. These measurements were then analyzed. An intraclass correlation coefficient was used to determine the reliability of this technique (Bland and Altman, 1986): this correlation coefficient was 0.90 (95% CI 0.75-0.96). A previously published study in infants has shown that the blood flow in the central retinal arteries are similar in both eyes. (Papacci et al., 1998a)

Statistical analyses were performed using MedCalc Version 11.6 (MedCalc Software bvba, Mariakerke, Belgium). Using Student's t-test, P values < 0.05 were considered significant. The normality of variables was determined using the D'Agostino-Pearson test (D'Agostino et al., 1990).

Results

A total of 247 babies were admitted to the department during the study period. Of these, 102 were suitable for recruitment, and their parents were approached for participation. Written consent was obtained for 31 infants. Ten babies were excluded because of LBW. Three infants were found to have spontaneous retinal hemorrhage, and 1 of these was excluded because of bilateral and diffuse retinal hemorrhage (Figure 2). Twenty newborn infants born at term (9 female infants and 11 male infants) were analyzed in this study. Their birth weights ranged from 2500 to 4,310 g (mean, 3,342 (523) g), and their gestational ages ranged from 37 to 41.6 weeks (mean, 39.2 (1.4) weeks). The retinal arteriole diameters were 66.8–123.0 μm (mean, 85.5 (14.3) μm), and the venule diameters were 102.0–167.8 μm (mean, 130.0 (16.0) μm). Table 1 compares the retinal arteriole and venule measurements in the term infants. The size of the retinal arterioles was significantly smaller than that of the retinal venules (85.5 vs. 130.0 μm ; $p < 0.00001$). The mean AVR was 0.66 (95% CI 0.62–0.70). The coefficient of correlation between the retinal arteries and veins was 0.56 (Figure 3). There were no differences between male and female infants, as shown in Table 2. These results were then compared to published retinal microvasculature measurements from children and adults (Table 3).

Discussion and Conclusions

Studies of retinal vasculature in infants have mainly focused on premature infants and ROP (Grunwald et al., 2009; Johnson et al., 2007). To our knowledge, this is the first study to investigate measurements of retinal microvasculature using digital retinal imaging in NBW term infants. These measurements could be used as a baseline for future studies that investigate the effects of birth weight and other neonatal conditions on retinal microvasculature. Previous

studies have shown a strong correlation between LBW and retinal vasculature size in older children (Cheung et al., 2008; Hellstrom et al., 1997; Mitchell et al., 2008; Sun et al., 2009; Tapp et al., 2007), adolescents (Gopinath et al., 2010), and adults (Chapman et al., 1997; Hellstrom et al., 2004; Liew et al., 2008a). However, no published studies have utilized the baseline measurements of infant retinal vasculature for comparison.

Compared to previously published results, retinal arteriole and venule diameters nearly double by the time a child is 6 years old (Mitchell et al., 2008). Over the same period of time, the body weight of the infant would have increased by more than fivefold (WHO, 2006). In young children and healthy adults, the retinal arteriole remains smaller than the retinal venule (Hughes et al., 2009; Mitchell et al., 2008; Wong et al., 2004a). The AVR is an important measure of retinal microvasculature that combines information from both arterial and venous system and has the advantage of controlling for magnification differences between camera lenses and refractive errors (Liew et al., 2007). Our study demonstrated the AVR in an NBW infant to be 0.66 and this ratio increase in adults to 0.89 (Wong et al., 2004a). The available data suggest that while retinal venule diameter remains approximately the same from the age of 6 onwards, the retinal arteriole diameter continues to grow from infancy to adulthood resulting in an increased AVR in a healthy adult (Mitchell et al., 2008; Wong et al., 2004a). The significance of this finding is that deviation of AVR from a normal value, in particular a lower AVR, has been associated with increased risk of stroke (Wong et al., 2001), hypertension (Wong et al., 2004b) and cardiovascular disease (Wang et al., 2006).

One of the main challenges that we faced in the newborn term infants in our study was spontaneous retinal hemorrhage. Three of the 21 patients (14%) in our cohort were found to have spontaneous retinal bleeding, and one had to be excluded because of diffuse bilateral bleeding, as

shown in Figure 2. The hemorrhage rendered measurements difficult and inaccurate.

Spontaneous retinal hemorrhage is a well-recognized event in healthy newborns. The percentage of full-term infants who develop spontaneous retinal hemorrhage at birth ranges from 10 to 30% (Hughes et al., 2006; Kaur and Taylor, 1990). The cause of this phenomenon is unknown. It does not require any treatment and resolves spontaneously. This condition is not related to the mode of childbirth (Hughes et al., 2006; Papacci et al., 1998b).

Another limitation of our study is our inability to account for any refractive errors that could have influenced the measurements (Cheung et al., 2008). In adults' and children's eyes, corrections can be applied to compensate for inaccuracies in the measurements of retinal structure that occur because of refractive error; these corrections require ocular biometric measurements, including axial length, anterior chamber depth, lens thickness, vitreous chamber depth parameters, and keratometry (measurements of the curvature of the anterior surface of the cornea) (De Silva et al., 2006). These measurements can easily be acquired in adults and young children. However, these calculations are more challenging in infants due to the continued growth of the eye and the inability of infants to remain still while these measurements are performed (De Silva et al., 2006).

The sample size in this study was limited, but we believe that the data obtained could be used as a baseline for future studies that investigate the role of retinal microvascular abnormalities in the development of cardiovascular diseases. We hope to perform a similar analysis in a cohort of LBW full-term infants in the future. **Results shown in table 3 were all obtained from different retinal cameras. Ideally, comparisons of retinal microvasculature between various age groups should be carried out using images produced by a similar retinal camera. However, the currently available retinal camera for use neonates is not**

suitable for children and adults. Perhaps in the future, when such technology is available, the comparisons will be more accurate.

In conclusion, the retinal microvasculature in human increases in diameter as an infant grows. The retinal venule stops growing in childhood whereas the retinal arteriole continues to grow until adulthood. The net result is an increase in AVR .Deviation from a normal AVR, particularly a low AVR, has been associated with an increased risk of vascular diseases.

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Figure Legends

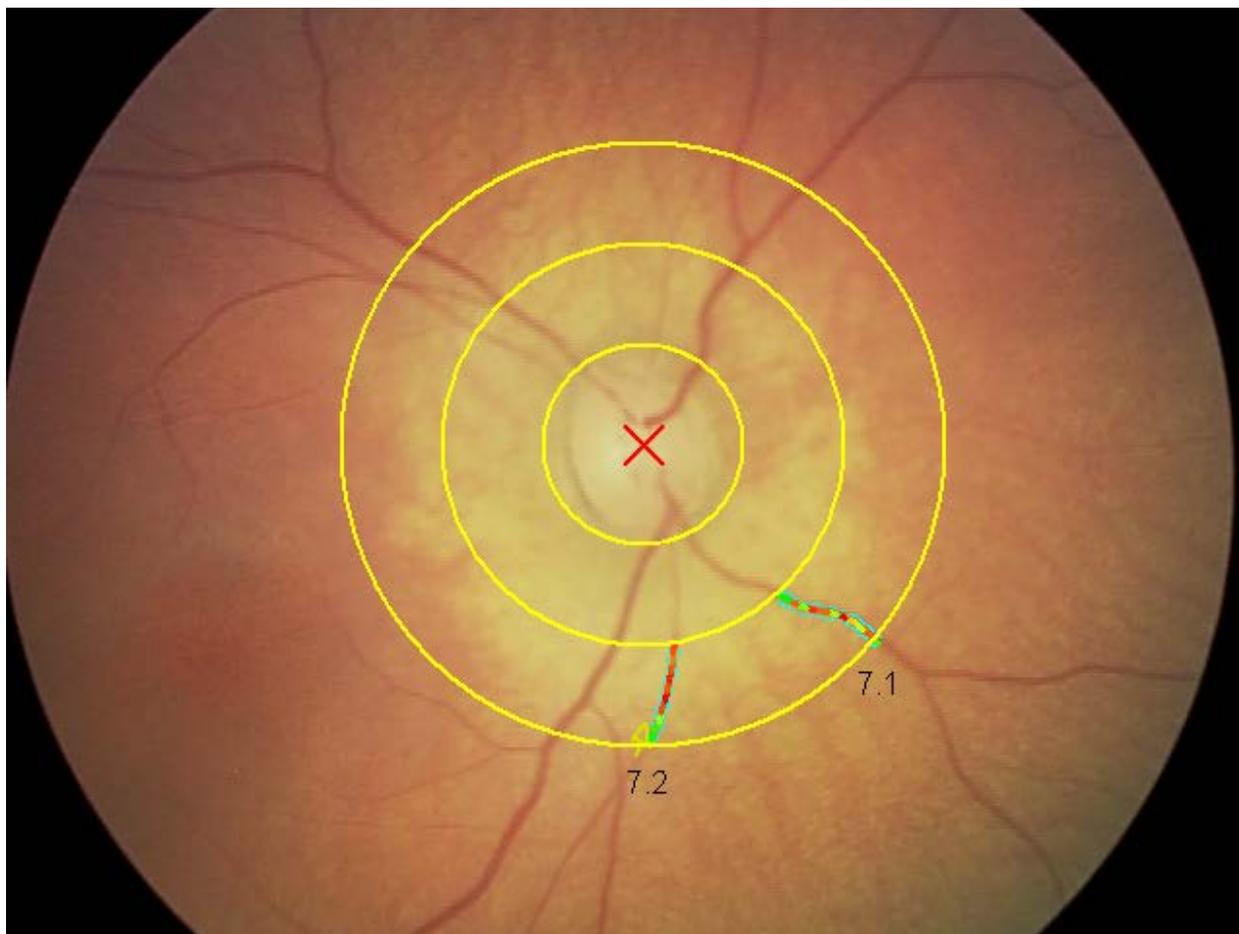


Figure 1. Retinal image from a newborn infant showing the identification and measurement of retinal vessels 0.5 to 1.0 disc diameters from the margin of the optic disc

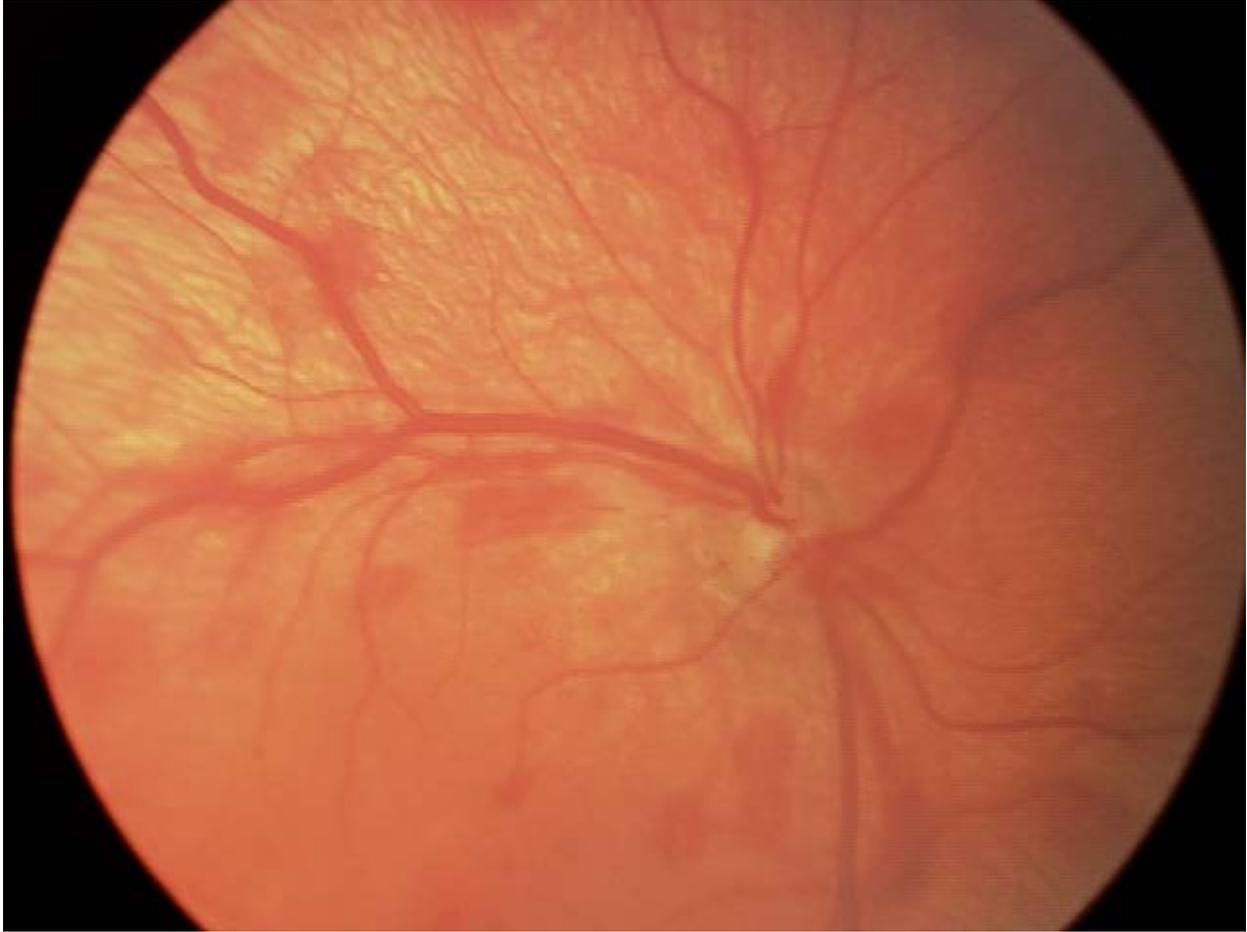


Figure 2. Spontaneous retinal hemorrhage in a full-term infant

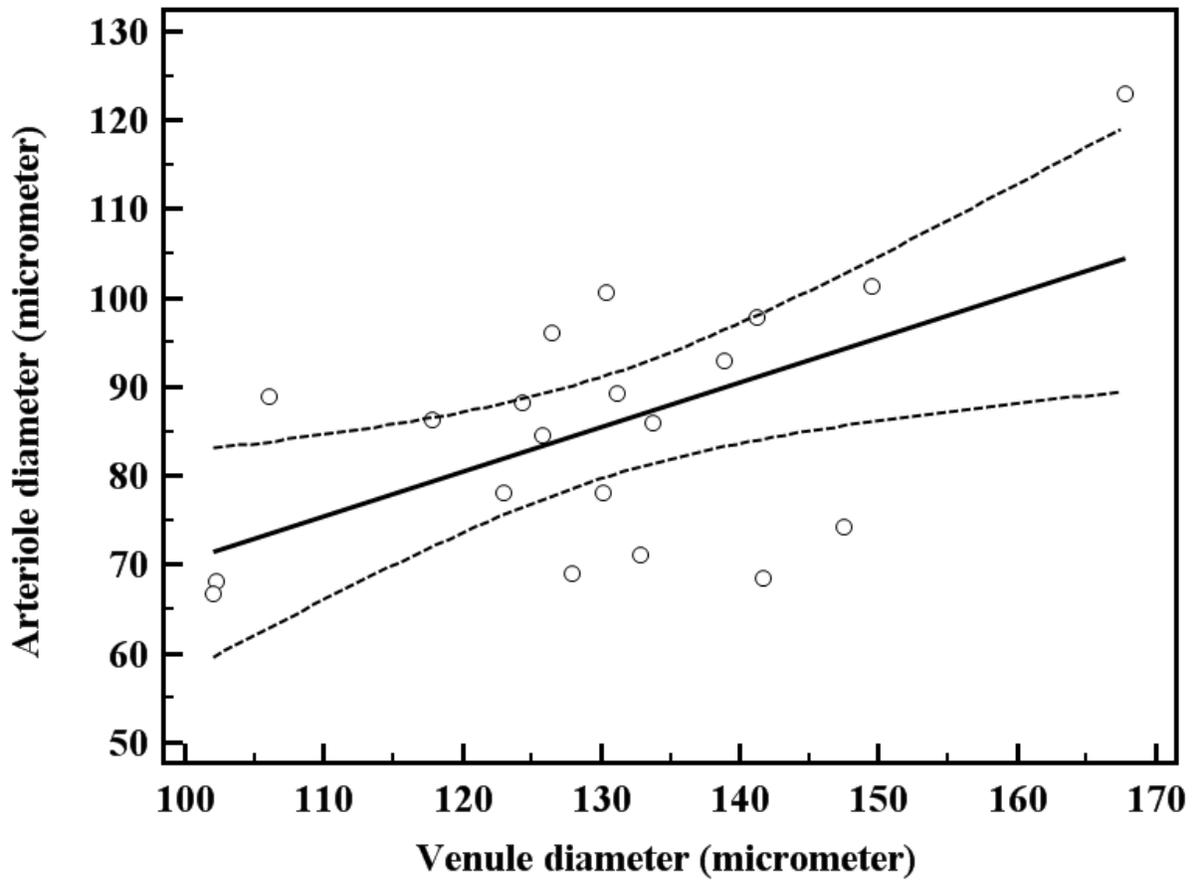


Figure 3. Graph showing the correlation between retinal arteriole and venule diameters in normal-weight, full-term infants. **Increase in retinal arteriole diameter is associated with an increase in retinal venule diameter.**

Table 1. Summary statistics comparing retinal arterioles and venules in normal-weight infants

Variable	Venule diameter	Arteriole diameter
Number of infants	20	20
Diameter (µm)	102.0-167.8	66.8-123.0
Mean (µm)	130.0± 16.0	85.5± 14.3
95% CI for the mean	122.5 to 137.5	78.8 to 92.2
Median (µm)	130.2	86.2
95% CI for the median	124.5 to 138.0	75.0 to 92.9
D'Agostino-Pearson test	Normal distribution (P=0.6289)	Normal distribution (P=0.1918)

Table 2. Comparison of measurements between male and female infants

Variables	Female	Male	p value
Number	9	11	
Birth weight (g)	3478(508)	3232(532)	0.308
Length (cm)	50.4(1.9)	49.4(3.0)	0.413
Head circumference (cm)	34.4(0.7)	34.7(1.7)	0.634
Arteriole diameter (μm)	88.3(16.9)	83.2(12.2)	0.437
Venule diameter (μm)	137.0(18.0)	124.3(12.2)	0.078
Arteriovenous ratio	0.64(0.10)	0.67(0.1)	0.591

Table 3 . Retinal microvasculature measurements in infants, children and adults.

Age	Mean retinal arteriole diameter (μm)	Mean retinal venule diameter (μm)	Arteriovenous ratio
Newborn term infant	85.5	130.0	0.66
Children (6 years) *	165.6	232.0	NA
Adults (43-84 years)**	202.3	227.2	0.89

* Mitchell et al., 2008 ,**Wong et al., 2004 , NA –not available