Relationship between the retinal microvasculature and renal volume in low-birth-weight babies

Corresponding author: Dr. Yogavijayan Kandasamy FRACP

Department of Neonatology

The Townsville Hospital, 100 Angus Smith Drive, Douglas

Queensland 4814, Australia

Tel: 61747962989 Fax: 61747962981

Email: Yoga_Kandasamy@health.qld.gov.au

Co authors: Prof Roger Smith FRACP, Assoc. Prof Dr Ian MR Wright FRACP

Affiliation: Mother and Babies' Research Centre

University of Newcastle

John Hunter Hospital,

Locked Bag 1, Hunter Region Mail Centre,

Newcastle, New South Wales 2310, Australia

ABSTRACT

Objective: We carried out a study to assess whether the development of the retinal microvasculature reflects nephron growth and therefore nephron number.

Study design: In our study, we determined the association between kidney volume (nephron number) and the retinal microvasculature of term LBW and normal birth weight (NBW) infants (11 LBW and 27 NBW).

Results: LBW infants had significantly larger retinal arteriolar and venular diameters (104.2 ± 21.4 vs. 87.0 ± 12.7 μm; $p = 0.004$; 146.8 ± 19.5 vs. 128.0 ± 19.5 μm; $p = 0.01$, respectively) compared with NBW infants. LBW infants also had smaller mean renal volumes (9.3 ± 2.3 vs. 12.2 ± 3.1 ml; $p = 0.008$). There were negative correlations between retinal arteriolar and venular diameters and renal volumes ($r = –0.34$, $p < 0.05$; $r = –0.37$, $p < 0.05$, respectively).

Conclusion: The larger the kidney (and, by implication, the greater the nephron number), the smaller are the diameters of retinal arterioles and venules. Thus, the degree of dilation of the retinal microvasculature provides an indirect index of renal growth.

Keywords: retinal vessels, renal volume, neonate, low birth weight

2

Abbreviations:

Low birth weight (LBW**)**

Normal birth weight (NBW)

INTRODUCTION

Low birth weight (LBW; <2500 g)[1] constitutes a risk factor for adult renal disease[2–5]. Globally, more than 20 million LBW infants are born each year[6]. Birth weight is strongly correlated with total glomerular number and glomerular size in the postnatal kidney. Total glomerular number has a direct linear relationship with birth weight, whereas mean glomerular volume is inversely correlated with glomerular number[7]. LBW infants constitute a heterogeneous group of babies who may be premature, growth restricted (birth weight below the 10th centile), or both. The critical window of the final stages of kidney development spans from 32 to 35 weeks of gestational age, and no new nephrons are formed after 36 weeks. Nephron endowment is, at that point, fixed for life[3,8].

The retina provides a unique opportunity for the *in vivo* investigation of the human microcirculation. The eye, kidney, and blood vessels are distinct organs that share common features in many systemic diseases[9]. In fact, the eye has been described as a window to the kidneys[10]. Histopathological changes that occur in both retinal and renal microcirculation in many clinical conditions are well recognized[9,10]. Studies have shown that retinal microvascular diameters are predictive of renal function[11–13]. In patients that have chronic kidney disease, the ratio of retinal arteriole to the venule lumen diameter has been found to be lower than that of matched controls[14]. The appearance of the retinal microvasculature is thus believed to be indicative of systemic microvasculature status, including the microvasculature of the kidneys.

Studies that have shown a link between LBW and reduced nephron endowment have mainly been based on autopsy findings[7,15,16]. Furthermore, the

4

results linking changes in the retinal microvasculature with renal function are predominantly from adult subjects[11–13]. Therefore, we performed an *in vivo* study on LBW infants to determine whether kidney volume is associated with changes in the retinal microvasculature.

METHODS

Participants

This cross-sectional study was performed in the Department of Neonatology, The Townsville Hospital, Queensland, Australia, 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 over a 12-month period. 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. Only babies who were born at term (i.e. after 37 weeks of gestation) were included in the study. Based on birth weight, infants were classified as term LBW or normal birth weight (NBW) (2500–4500 g). Infants who needed **oxygen and/or** respiratory support or surgery, those that were born with a large birth weight (>4500 g), and those born to mothers with gestational diabetes were excluded, as were those with syndromes, prematurity, or chromosomal abnormalities. All assessments were performed within the first seven days of life.

Retinal microvasculature measurements

Digital images of both retinas were obtained using a retinal camera (RetCam, Massie Laboratories, Dublin, CA, USA**).** This device is a contact retinal camera and the measurements could be affected by the cornea pressure .Hence, the measurements were only carried out by a trained operator (YK) and only minimal pressure was applied during assessment. The measurements of retinal vessel diameters were then obtained using a predetermined protocol that first involved the identification of retinal vessels located 0.5–1 disc diameters from the margin of the optic disc. We provided pain relief using oral sucrose and local anesthetic agents during the eye examination[17]. Pupillary dilation was carried out using cyclopentolate and phenylephrine ophthalmic drops. Video and still retinal images with the optic disc in the center were obtained and the sharpest image was then chosen to measure the vessels. Papacci et al. showed that the blood flow in the central retinal artery of infants is similar in both eyes[18]. Vessel diameter, computed as the distance between the walls within the vessel, was measured using semi-automated software (Vesselmap, IMEDOS GmbH, Jena, Germany)[19–21]. Thus, the caliber of directly viewed vessels was determined by the size of the red cell column, since the vessel walls and peripheral plasma layer were almost transparent[22]. Vessels were measured in each eye, and the largest venule and arteriole of each patient were determined and recorded. An intraclass correlation coefficient was used to determine the reliability of this technique[23]; the correlation coefficient was 0.90 (95% confidence interval: 0.75–0.96).

Renal sonography

6

All renal sonograms were obtained using the Philips IU22 Ultrasound System (Philips Healthcare, Andover, MA, USA) with a 5–8 MHz transducer. The bilateral longitudinal renal length (L), maximal anteroposterior (AP) diameter, and transverse diameter (W) were then measured. To avoid inter-observer errors during scanning and measuring, all scanning was performed by the same sonographer. Kidney volume (KV) was estimated according to the formula $KV = 0.523 \times L \times W \times AP$[24]. The mean volumes of the right and left kidneys ([Right KV + Left KV] / 2) were then calculated.

Statistical analysis

Statistical analyses were performed using MedCalc Version 11.6 (MedCalc Software, Mariakerke, Belgium). Data are expressed as mean ± SD or as median [Inter quartile range] where appropriate. Differences were considered to be significant at $p < 0.05$.

RESULTS

A total of 524 patients were admitted to the department during the study period, of which 227 fulfilled the recruitment criteria. Forty-three infants were recruited, and complete eye and ultrasound scan data were available for 38 infants (11 LBW and 27 NBW; 17 male and 21 female).The mean birth weight for NBW and LBW were 3348±481 g and 2265 ± 183 g respectively. There were no significant difference in the mean gestational age (39.1±1.4 weeks vs.38.3±1.0 weeks; p= 0.09) and the median post natal age (4.0[3.0-7.0] days vs. 5.0[3.0-7.3] days; p =0.8) between NBW and LBW infants.

LBW infants had significantly larger retinal arteriole diameter compared with NBW infants (104.2 ± 21.4 vs. 87.0 ± 12.7 μm; p = 0.004). The retinal venule

diameters was also larger in LBW cohort compared to NBW cohort (146.8 ± 19.5 vs. 128.0 ± 19.5 μm; $p = 0.01$). LBW infants also had smaller mean renal volumes (9.3 ± 2.3 vs. 12.2 ± 3.1 ml; $p = 0.008$). There were negative correlations between retinal arteriolar and venular diameters and renal volumes ($r = –0.34$, $p < 0.05$; $r = –0.37$, $p < 0.05$, respectively) (Figures 1 and 2). We also compared male and female infants but found no differences in kidney volumes (11.9 ± 3.0 vs. 10.9 ± 3.3 mL; $p = 0.34$), retinal arteriole diameters (88.5 ± 15.5 vs. 95.0 ± 18.5 μm; $p = 0.27$), or retinal venule diameters (129.3 ± 20.4 vs. 137.2 ± 21.0 μm; $p = 0.26$).

DISCUSSION

We found that the retinal vessel sizes correlated negatively with renal volume. In addition, retinal vessel diameters (arteriole and venule) were larger in LBW infants. There are many causes of intrauterine growth restriction, but the most common is uteroplacental insufficiency, which results in fetal hypoxia[25,26]. We postulate that the observed changes in the retinal vessels of our LBW cohort were the result of fetal hypoxia. This finding is most likely to be the result of direct retinal and kidney effects on blood flow, but there may also be secondary changes in the glomeruli of LBW individuals[7,15], mediated by vascular endothelial growth factor (VEGF).

Blood flow in the human retina is subject to autoregulation[27], a feature of the retina that is similar to that of the kidneys[9]. Retinal circulation is characterized by a low blood flow and a high level of oxygen extraction; the arteriovenous difference in pO_2 is approximately 40%[28]. Autonomic nerve endings do not extend into the intraocular segments of retinal blood vessels. Therefore, retinal arterial tone is largely regulated by local factors such as local variations in perfusion pressure, pO_2, pCO_2, and pH[28]. Hypoxia plays a major role in the process of angiogenesis in retinal

8

vessels[29]. The positive regulators of angiogenesis include the members of the VEGF family, angiopoietins, transforming growth factors, epidermal growth factor, platelet-derived growth factor, tumor necrosis factor-α, insulin-like growth factor, vascular endothelial-cadherin, interleukins, and members of the fibroblast growth factor family[29].

VEGF is an essential agent of angiogenesis in diseases of the eye[29]. Of the various types of VEGF present in humans, VEGF-A is the most crucial form for vasculogenesis[30]. Six different isoforms of VEGF-A have been identified in humans[30]. Five types of retinal cells have the capacity to produce and secrete VEGF: the retinal pigmented epithelium, astrocytes, Muller cells, endothelial cells, and ganglion cells[30]. We postulate that placental insufficiency results in fetal hypoxia and a change in the local levels of pO_2, pCO_2, and pH. This stimulates the retinal cells to excrete VEGF in order to overcome local hypoxia in the eye. VEGF promotes angiogenesis and vessel dilatation. The dilatation of retinal vessels could also be further enhanced to some extent by an increase in middle cerebral artery flow, which occurs in a hypoxic fetus[31]. Doppler flowmetry data from newborn babies have shown that an increase in blood flow in the middle cerebral and ophthalmic arteries is closely followed by an increase in blood flow in the central retinal artery[31].

Extensive changes occur in the circulation of growth-restricted LBW fetuses in response to intrauterine hypoxia. These changes, often referred to as 'redistribution' or the 'brain-sparing effect', reduce vascular resistance in those organs that are essential to fetal survival, such as the brain and coronary arteries[32,33]. Doppler studies have also shown that fetal hypoxia reduces cardiac output as well as the percentage of cardiac output directed towards the kidneys, thereby resulting in the reduction of renal perfusion, urine production, and amniotic fluid volume in growth-

restricted fetuses[32,33]. Autopsy findings from LBW infants have shown a reduction in glomerular number (and kidney volume) with compensatory glomerulomegaly compared with findings from NBW infants[15]. VEGF has also been found to be essential for glomerular and tubular hypertrophy and endothelial cell proliferation in response to nephron reduction[34]. In the human kidney, the VEGF receptors VEGFR-1 and VEGFR-2 are predominantly expressed on preglomerular, glomerular, and peritubular endothelial cells[34]. Figure 3 summarizes the relationship between fetal hypoxia, the retinal vasculature, and the glomeruli of the kidney.

The main limitation of this study is the number of subjects recruited. The consent rate was approximately 20%. Participation was voluntary, and parents were not expected to explain why they declined to participate. It is possible that the examination involved in the study might have appeared to be unpleasant for their infants. Another challenge that we faced in the study of newborn term infants was spontaneous retinal hemorrhage, which rendered measurements difficult and inaccurate. Spontaneous retinal hemorrhage is a well-recognized event in healthy term newborns[35]. The percentage of full-term infants who develop spontaneous retinal hemorrhage at birth ranges from 10% to 30%. The cause of this phenomenon is unknown, but it is not related to the mode of childbirth. Further, it does not require any treatment and resolves naturally.

CONCLUSION

The larger the kidney (and, by implication, the greater the nephron number), the smaller are the diameters of the retinal arterioles and venules. **The increase in retinal vessel diameters is possibly due to compensatory increase in cerebral blood flow in response to fetal hypoxia.** Therefore, we suggest that this association

shows that chronic hypoxia impairs renal nephrogenesis, as systemic blood flow is restricted in order to maintain cerebral oxygen delivery. Thus, the degree of dilation of the retinal microvasculature could provide an indirect index of renal growth in NBW and LBW infants. We propose that the assessment of the retinal microvasculature is a non-invasive tool to determine at-risk individuals using dilated retinal vessels in LBW infants as a proxy indicator of reduced nephron number.

Acknowledgements: Research grants available for this study were: The Townsville Hospital Private Practice Research and Education Grant, The Royal Australasian College of Physicians Research and Education Grant, and ANZ Queensland Children's Medical Research Grant.

Statements of interest: None

Reference

1. International Classification of Diseases and Related Health Problems.10th revision. Geneva: World Health Organization;1992.

2. Franke D, Volker S, Haase S et al. Prematurity, small for gestational age and perinatal parameters in children with congenital, hereditary and acquired chronic kidney disease. Nephrol Dial Transplant 2010;25:3918-3924.

3. Bagby SP Developmental origins of renal disease: should nephron protection begin at birth? Clin J Am Soc Nephrol 2009;4:10-13.

4. Hoy WE, Rees M, Kile E et al. Low birthweight and renal disease in Australian aborigines. Lancet 1998;352:1826-1827.

5. White SL, Perkovic V, Cass A et al. Is low birth weight an antecedent of CKD in later life? A systematic review of observational studies. Am J Kidney Dis 2009;54:248-261.

6. Organization UNCsFWH . Low Birthweight - Country, Regional and Global Estimates. UNICEF, New York. 2004

7. Hughson M, Farris AB, 3rd, Douglas-Denton R, Hoy WE, Bertram JF . Glomerular number and size in autopsy kidneys: the relationship to birth weight. Kidney Int 2003;63:2113-2122.

8. Shah MM, Sampogna RV, Sakurai H, Bush KT, Nigam SK . Branching morphogenesis and kidney disease. Development 2004;131:1449-1462.

9. Blum M, Saemann A, Wolf G .The eye, the kidney and microcirculation. Nephrol Dial Transplant 2010;26:4-6.

10. D'Souza YB, Short CD . The eye- a window on the kidney. Nephrol Dial Transplant 2009;24:3582-3584.

11. Cuspidi C, Dell'Oro R, Grassi G.Retinal arteriolar narrowing as marker of renal dysfunction: potential value and limitations. J Hypertens 2009;27:2162-2164.

12. Sabanayagam C, Tai ES, Shankar A, Lee J, Sun C, Wong TY. Retinal arteriolar narrowing increases the likelihood of chronic kidney disease in hypertension. J Hypertens 2009;27:2209-2217.

13. Ooi QL, Tow FK, Deva R et al. The microvasculature in chronic kidney disease. Clin J Am Soc Nephrol 2011;6:1872-1878.

14. Baumann M, Schwarz S, Kotliar K et al. Non-Diabetic Chronic Kidney Disease Influences Retinal Microvasculature. Kidney and Blood Pressure Research 2009;32:428-433.

15. Guignard JP, Drukker A. Why do newborn infants have a high plasma creatinine? Pediatrics 1999;103:e49.

16. Hoy WE, Douglas-Denton RN, Hughson MD, Cass A, Johnson K, Bertram JF. A stereological study of glomerular number and volume: preliminary findings in a multiracial study of kidneys at autopsy. Kidney Int Suppl 2003;S31-37.

17. Kandasamy Y, Smith R, Wright IM, Hartley L. Pain relief for premature infants during ophthalmology assessment. J Aapos 2011;15:276-280.

18. Papacci P, Romagnoli C, Favuzzi A et al. Doppler ultrasound of blood flow velocities in ophthalmic and central retinal arteries during the early neonatal period. Am J of Opthalmol 1998;126:691-697.

19. Grunwald L, Mills MD, Johnson KS et al. The rate of retinal vessel dilation in severe retinopathy of prematurity requiring treatment. Am J Ophthalmol 2009;147:1086-1091

20. Johnson KS, Mills MD, Karp KA, Grunwald JE. Quantitative analysis of retinal vessel diameter reduction after photocoagulation treatment for retinopathy of prematurity. Am J Ophthalmol 2007;143:1030-1032.

21. Kandasamy Y, Smith R, Wright IM. Retinal microvasculature measurements in full-term newborn infants. Microvasc Res 2011;82:381-384.

22. Archer, DB, Gardiner, TA, Stitt, AW. Retinal Vascular Disease. Heidelberg: Springer; 2010: p 23.

23. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307-310.

24. Hricak H, Slovis TL, Callen CW, Callen PW, Romanski RN. Neonatal kidneys: sonographic anatomic correlation. Radiology 1983;147:699-702.

25. Hendrix N, Berghella V. Non-placental causes of intrauterine growth restriction. Semin Perinatol 2008;32:161-165.

26. Sankaran S, Kyle PM. Aetiology and pathogenesis of IUGR. Best Pract Res Clin Obstet Gynaecol 2009;23:765-777.

27. Blum M, Bachmann K, Wintzer D, Riemer T, Vilser W, Strobel J. Noninvasive measurement of the Bayliss effect in retinal autoregulation. Graefes Arch Clin Exp Ophthalmol 1999;237:296-300.

28. Hardy P, Beauchamp M, Sennlaub F et al. New insights into the retinal circulation: Inflammatory lipid mediators in ischemic retinopathy. Prostaglandins, Leukotrienes and Essential Fatty Acids 2005;72:301-325.

29. Siemerink MJ, Augustin AJ, Schlingemann RO. Mechanisms of ocular angiogenesis and its molecular mediators. Dev Ophthalmol 2010;46:4-20.

30. Holmes DI, Zachary I. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. Genome Biol 2005;6:209.

31. Kandasamy Y, Smith R, Wright IM, Hartley L .Relationship between birth weight and retinal microvasculature in newborn infants. J Perinatol 2011; 9: 22. doi: 10.1038/jp.2011.118. [Epub ahead of print].

32. Stigter RH, Mulder EJH, Bruinse HW, Visser GHA. Doppler studies on the fetal renal artery in the severely growth-restricted fetus. Ultrasound in Obstetrics and Gynecology 2001;18:141-145.

33. Pearce W . Hypoxic regulation of the fetal cerebral circulation. J Appl Physiol 2006;100:731-738.

34. Schrijvers BF, Flyvbjerg A, De Vriese AS .The role of vascular endothelial growth factor (VEGF) in renal pathophysiology. Kidney Int 2004;65:2003-2017.

35. Hughes LA, May K, Talbot JF, Parsons MA. Incidence, distribution, and duration of birth-related retinal hemorrhages: a prospective study. J Aapos 2006;10:102-106.

Figure legends

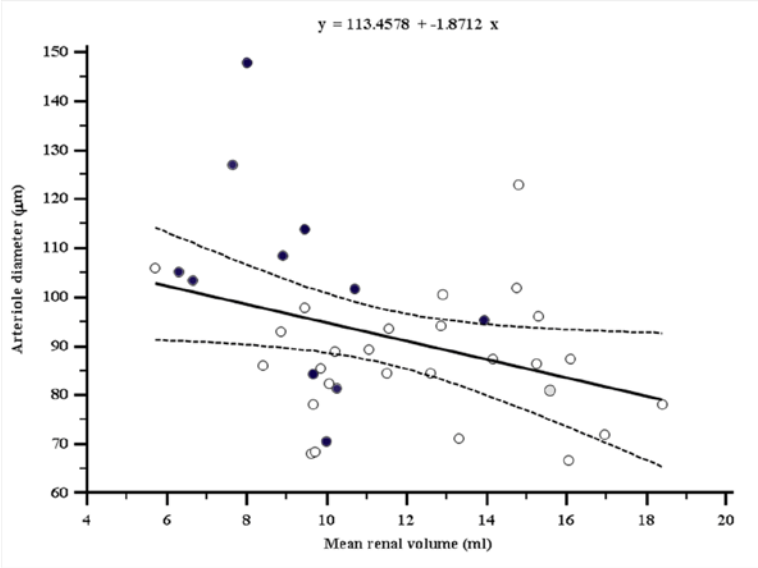

Figure 1. The relationship between retinal arteriole diameter and renal volume. Infants that had smaller renal volumes showed larger retinal arteriole diameters. There was also a significant decline in arteriolar diameter as renal volume increased ($r = –0.34$, $p < 0.05$). (● for Low birth weight infants)

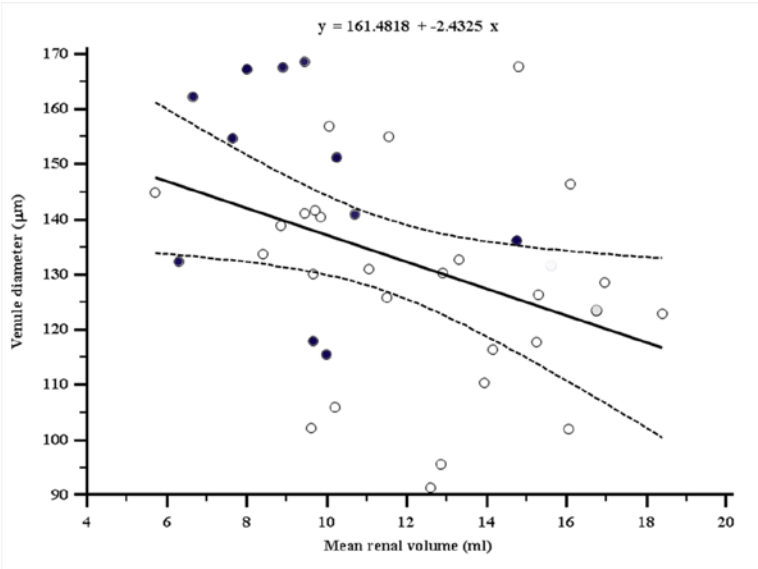

Figure 2. The relationship between retinal venule diameter and mean renal volume. Infants that had lower birth weights and smaller kidney volumes showed larger retinal venule diameters. There was also a significant decline in venule diameter as renal volume increased ($r = –0.37$, $p < 0.05$). (● for Low birth weight infants)

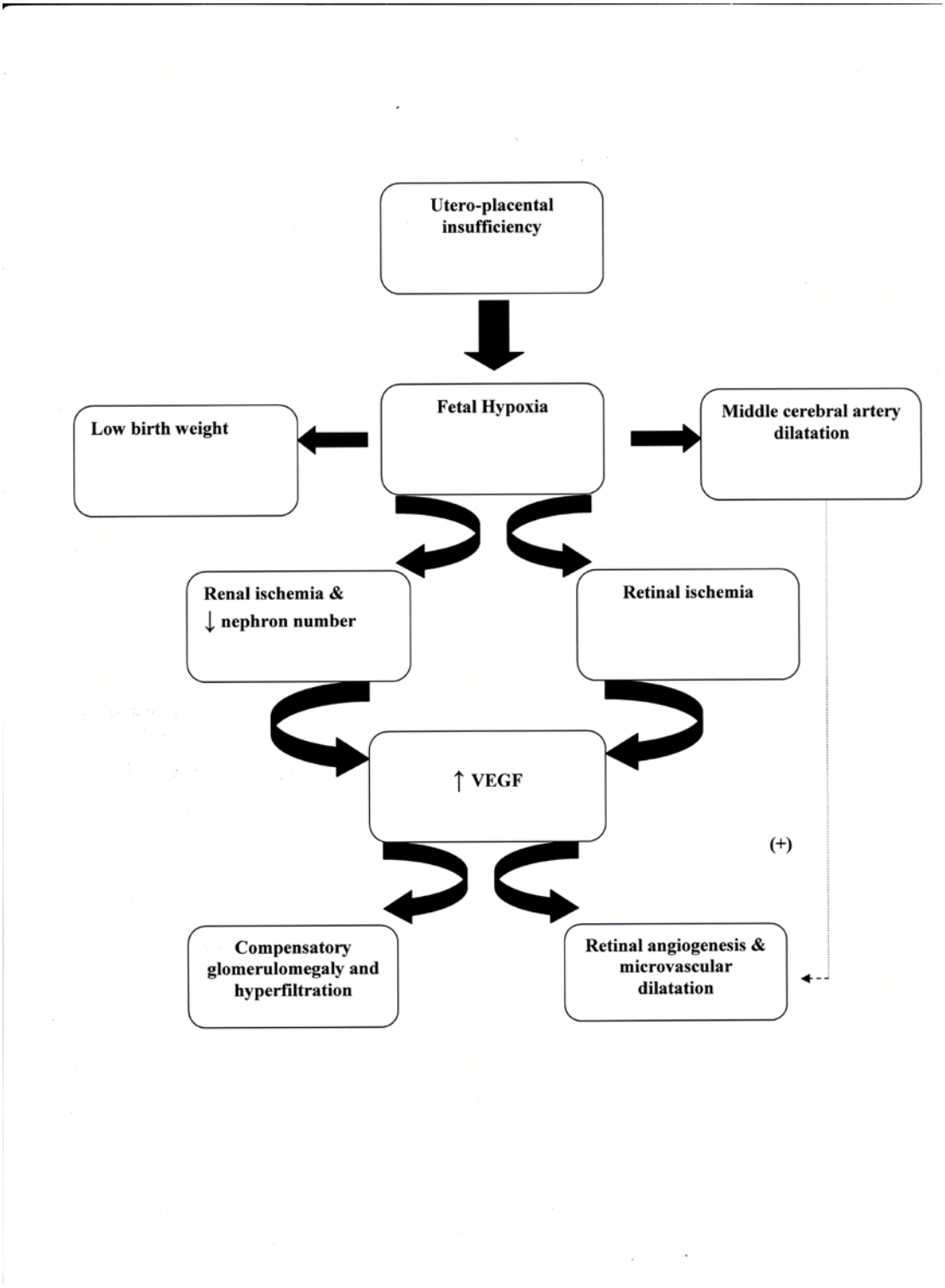

Figure 3. The effect of fetal hypoxia on the renal and retinal microvasculature.

Fetal hypoxia causes retinal vessel dilatation and compensatory glomerulomegaly and hyperfiltration. VEGF is believed to be one of the mediators underlying this process.

The dilatation of retinal vessels may also be secondary to increased middle cerebral artery flow. ((+) = contributes, ↑= increase, ↓= decrease)