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REVIEW

Retinal vascular image analysis as a potential screening tool for cerebrovascular disease: a rationale based on homology between cerebral and retinal microvasculatures

Niall Patton,[1] Tariq Aslam,[2] Thomas MacGillivray,[3] Alison Pattie,[4] Ian J. Deary[4] and Baljean Dhillon[1]

[1]*Princess Alexandra Eye Pavilion, Chalmers Street, Edinburgh, UK*
[2]*Manchester Royal Eye Hospital, Oxford Road, Manchester, UK*
[3]*Welcome Trust Clinical Research Facility, Western General Hospital, Crewe Road, Edinburgh, UK*
[4]*School of Philosophy, Psychology and Language Sciences, University of Edinburgh, UK*

Abstract

The retinal and cerebral microvasculatures share many morphological and physiological properties. Assessment of the cerebral microvasculature requires highly specialized and expensive techniques. The potential for using non-invasive clinical assessment of the retinal microvasculature as a marker of the state of the cerebrovasculature offers clear advantages, owing to the ease with which the retinal vasculature can be directly visualized *in vivo* and photographed due to its essential two-dimensional nature. The use of retinal digital image analysis is becoming increasingly common, and offers new techniques to analyse different aspects of retinal vascular topography, including retinal vascular widths, geometrical attributes at vessel bifurcations and vessel tracking. Being predominantly automated and objective, these techniques offer an exciting opportunity to study the potential to identify retinal microvascular abnormalities as markers of cerebrovascular pathology. In this review, we describe the anatomical and physiological homology between the retinal and cerebral microvasculatures. We review the evidence that retinal microvascular changes occur in cerebrovascular disease and review current retinal image analysis tools that may allow us to use different aspects of the retinal microvasculature as potential markers for the state of the cerebral microvasculature.

Keywords anatomical and physiological homology; cerebral microvasculature; cerebrovascular disease; digital image analysis; retinal microvasculature.

Introduction

The retina and the brain are highly metabolically active tissues with large demands on metabolic substrates via specialized vascular networks. Embryologically, the retina is an extension of the diencephalon, and both organs share a similar pattern of vascularization during development (Risau, 1997; Hughes et al. 2000; Dorrell et al. 2002; Lutty et al. 2002). There is a close anatomical correlation between both the macrovascular and the microvascular blood supply to the brain and the retina, and both vascular networks share similar vascular regulatory processes (Lassen, 1964; Hardy et al. 1997; Delaey & Van de Voorde, 2000b).

Assessment of the cerebral vasculature is important in determining an individual's risk of particular cerebrovascular diseases, such as vascular dementia (Mielke & Heiss, 1998; Varma et al. 2002; Yoshikawa et al. 2003) and stroke (Sadek & Hammeke, 2002; Powers & Zazulia, 2003). Investigative techniques currently used include transcranial Doppler ultrasound, positron emission tomography (PET), single-photon tomography (SPECT) and functional neuroimaging using magnetic resonance

Correspondence
Dr Niall Patton, MRCOpth., Lions Eye Institute, 2 Verdun Street, Nedlands, WA 6009, Western Australia. E: niallpatton@hotmail.com

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imaging (fMRI). fMRI techniques utilize either blood oxygen level-dependent (BOLD) contrast (Ogawa et al. 1992), dynamic contrast-enhanced imaging (Calamante et al. 1999) or arterial spin labelling (ASL) (Detre et al. 1992) and have become increasingly prevalent over the past decade. These techniques have provided invaluable tools in advancing our understanding of cerebrovascular pathophysiology (Kessler, 2003). However, these techniques are often expensive and available only in specialized centres, and therefore are not suitable candidates for more widespread screening of patients at risk of cerebrovascular disease. A simpler, more accessible technique is required.

Retinal digital image analysis may indirectly provide such a technique. Owing to the homology between the retinal and cerebral microvasculatures, changes in the retinal vasculature may reflect similar changes in the cerebral vasculature. The potential for using the retinal vasculature as a marker of the state of the cerebrovasculature offers clear advantages, owing to the ease with which the retinal vasculature can be directly visualized *in vivo*, and also photographed because of its essential two-dimensional nature. The use of retinal digital image analysis has become increasingly common over the past decade, and offers increasingly sophisticated techniques to analyse different aspects of retinal vascular topography, such as the widths of retinal microvessels. It has long been known that vascular topography, including the angles at which blood vessels bifurcate and the relationship between the widths of parent to daughter blood vessels at vascular junctions, is not necessarily random, but is optimized in order to minimize physical properties such as shear stress across any vascular network (Murray, 1926a,b; Zamir, 1976; Zamir et al. 1979; Sherman, 1981; Zamir & Medeiros, 1982). Changes in this optimal geometrical topography are known to occur in certain vascular conditions (Stanton et al. 1995b; Chapman et al. 2002). Analysis of these vascular properties through digital image analysis may offer an opportunity to study the potential for retinal microvascular abnormalities to act as markers of cerebrovascular pathology.

In this review, we outline the anatomical and physiological homology between the retinal and cerebral microvasculatures. We review the evidence that retinal microvascular changes occur in cerebrovascular disease and review recent advances in retinal image analysis tools that may allow us to consider retinal digital image analysis as a potential screening tool for cerebrovascular disease.

Comparative microvascular anatomy

Cerebral capillaries create a rich anastomotic vasculature throughout the brain, the density of which varies according to the activity and metabolic demand of the particular brain region, e.g. microvascular density in the grey matter of the brain is three times greater than that observed in the white matter, and sensory centres are more richly supplied than motor centres (Gjedde & Diemer, 1985; Klein et al. 1986). Retinal capillary density is greater in the central retina, but decreases towards the retinal periphery. The extreme retinal periphery is avascular. Specific regions of the retina identified as dominating the oxygen requirements of the retina include the inner segments of the photoreceptors (Linsenmeier, 1986) and the inner and outer plexiform layers (Yu & Cringle, 2001; Cringle et al. 2002).

The retinal circulation is a relatively low-flow (Alm & Bill, 1973) and high-oxygen-extraction system (Tornquist & Alm, 1979). The retinal capillary microvasculature has two distinct beds: the superficial capillary layer in the nerve fibre/ganglion cell layer, and the deeper capillary layer extending into the inner nuclear and outer plexiform layers (Toussaint et al. 1961). These capillaries have a diameter of approximately 5–6 μm (smaller than the cerebral capillaries) (Leber, 1903; Cogan & Kuwabara, 1984). Unlike the cerebral microvasculature, which has more abundant collateral channels, retinal blood vessels are end arteries without anastomotic connections, and therefore occlusion of these vessels leads to destruction of the inner layers of the retina (Yanoff & Fine, 1989). Unlike cerebral arterioles, retinal arterioles often show 90° vascular branching patterns (Cogan & Kuwabara, 1984).

Both the inner retinal and the cerebral circulation are 'barrier' circulations

The inner retinal and cerebral microcirculations share anatomical and physiological properties owing to their similar functions acting as 'barrier' endothelia. This barrier function serves to maintain the neuronal milieu from exogenous toxins, to buffer variations in blood composition and to restrict the transfer of small hydrophilic and large molecules and haematogenous cells in the brain and retina (Lightman et al. 1987; Bradbury & Lightman, 1990; Tornquist et al. 1990; Pardridge, 1995). This barrier consists of both mechanical and metabolic components (Fig. 1). The mechanical barrier is primarily attributed to the presence of tight junctional intercellular complexes

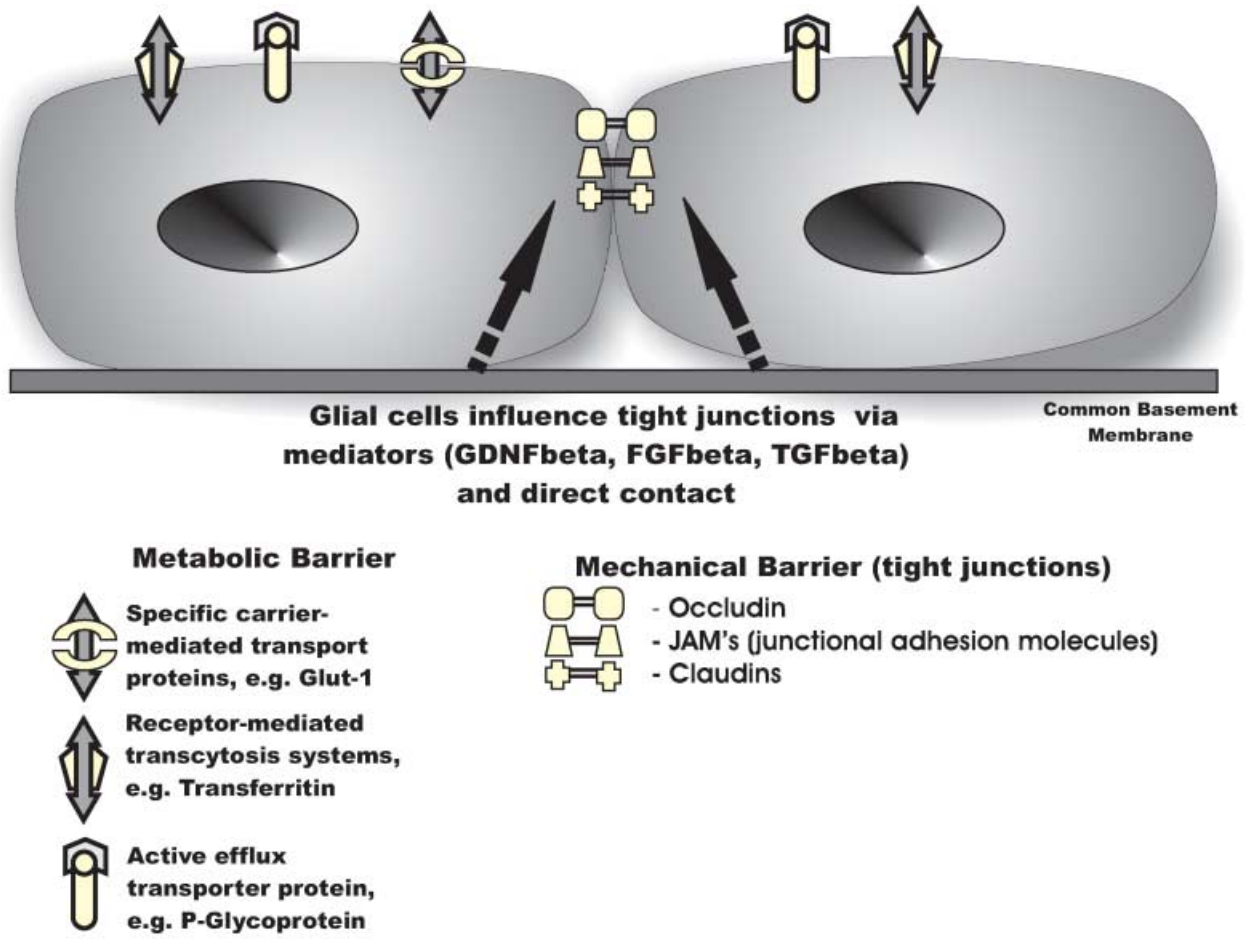

Fig. 1 Schematic diagram of the mechanical and metabolic components of the blood–brain and blood–retinal barriers and the influence of glial cells on these barriers. The mechanical component consists of the presence of apical (luminal) tight junctions composed of proteins such as occludin, claudins and junctional adhesion molecules (JAMs), often in conjunction with submembranous tight-junction-associated proteins, such as zonula occludens. The metabolic component consists of transport proteins, including GLUT-1, P-glycoprotein and transferritin.

between the endothelial cells of both the cerebral and the retinal vasculature on their luminal aspect. Barrier endothelia are no longer considered as static lipid membrane barriers, but as dynamic interfaces, with specific and selective membrane transport acting as a metabolic barrier (Cornford, 1985). The presence of specific carrier-mediated transport proteins is a feature of both the blood–brain barrier and the inner blood–retinal barrier (Betz et al. 1983; Bradbury, 1985; Tornquist & Alm, 1986; Neuwelt, 1989) (see below).

Constituents of the cerebral and retinal microvasculatures

Endothelial cells

The endothelial cells of the cerebral and retinal capillaries form a single layer around the capillary lumen. They are non-fenestrated and possess tight junctional intercellular complexes between the endothelial cells of both the cerebral and the retinal vasculature on their luminal aspect (Brightman, 1989; Pardridge, 1993; Wolburg & Lippoldt, 2002; Vorbrodt & Dobrogowska, 2003). Tight junctional complexes are composed of several proteins, including occludin (Morcos et al. 2001; Wolburg & Lippoldt, 2002), junctional adhesion molecules (JAM) (Martin-Padura et al. 1998) and claudins (Tsukita & Furuse, 1999; Morcos et al. 2001) (Fig. 1). Transmembrane proteins are often found in conjunction with submembranous tight junction-associated proteins [zonula occludens (ZO-1, ZO-2, ZO-3)] (Stevenson et al. 1986; Watson et al. 1991; Wolburg & Lippoldt, 2002). Tight junctions form the mechanical component of the blood–brain and inner blood–retinal barriers.

Endothelial cells lack fenestrations and have a paucity of pinocytotic vesicles (Coomber & Stewart, 1986; Bertossi et al. 1997; Farkas & Luiten, 2001). They are rich in mitochondria and the presence of specific carrier-mediated transport proteins is a feature of both vasculatures (Betz et al. 1983; Bradbury, 1985; Tornquist & Alm, 1986; Neuwelt, 1989) (Fig. 1). These transport mechanisms form an important role in the metabolic component of the blood–brain and blood–retina barriers and include GLUT1 and GLUT3 (for glucose transportation) (Maxwell et al. 1989; Takata et al. 1992; Cunha-Vaz, 1997; Badr et al. 1999; Mann et al. 2003), and specific amino acid protein transport systems (Tornquist & Alm, 1986; Tornquist et al. 1990; Mann et al. 2003). These metabolic markers of barrier endothelia provide specific carrier-mediated transport of nutrients such as glucose and amino acids across the tight junctions, as well as enzymatic degradation of molecules crossing the blood–brain and blood–retinal barriers. In addition, it has been postulated that the asymmetric distribution of plasma membrane proteins on the endothelia (luminal vs. abluminal) creates a polarized endothelium, which helps to create an electrical resistance to permeability (Crone & Olesen, 1982).

Pericytes

The pericyte surrounds the capillary endothelial cell. Pericytes are embedded within a common basement membrane with the endothelial cell, provide structural support to the microvasculature and are required for the establishment of the blood–brain and blood–retina barriers (LeBeux & Willemot, 1980; Farrell et al. 1987; Frey et al. 1991; Risau et al. 1992; Healey & Wilk, 1993; Song et al. 1993; Martin et al. 2000; Ramsauer et al. 2002). Retinal pericytes are known to cover more of the retinal endothelial network than their cerebral counterparts (Cogan & Kuwabara, 1984; Frank et al. 1987b, 1990) (Fig. 2). Pericytes are the capillary counterparts of vascular smooth muscle cells, containing α-smooth muscle actin and having contractile properties (LeBeux & Willemot, 1980; Herman & D'Amore, 1985; Joyce et al. 1985a,b). Cerebral pericytes also have a phagocytic role, which may operate as a 'second line of defence' at the boundary between blood and brain (Jordan & Thomas, 1988; Tagami et al. 1990; Thomas, 1999; Rucker et al. 2000).

Basement membranes

As well as providing structural support to the microvasculature, other functions of the basement membranes include influencing endothelial function, filtration of macromolecules and cell adhesion (Perlmutter & Chui, 1990). Cerebral basement membrane is the site of deposition of β-amyloid peptide in Alzheimer's disease (Perlmutter, 1994; Farkas & Luiten, 2001), and is also thickened in Parkinson's disease and experimentally in spontaneously hypertensive rats (Tagami et al. 1990).

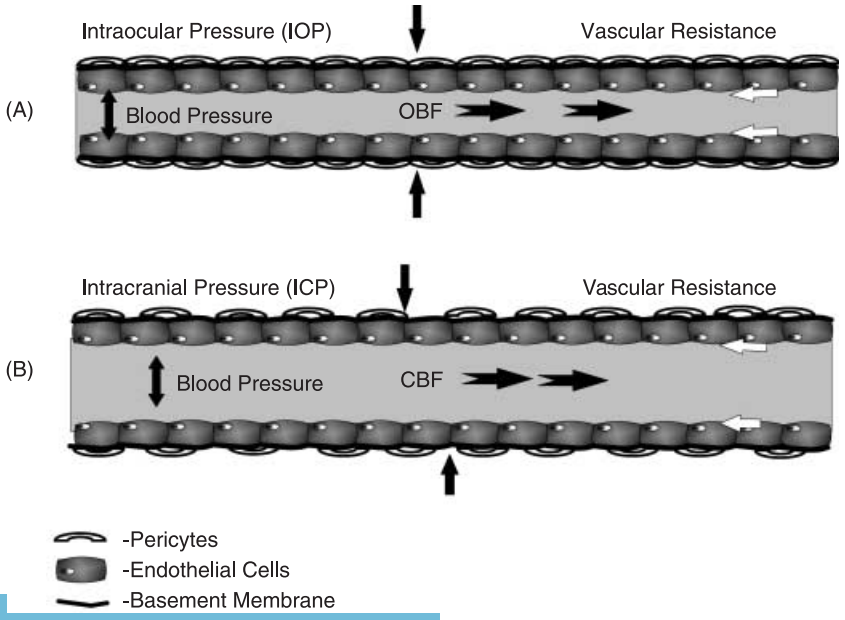

Fig. 2 Schematic diagram of (a) the retinal and (b) the cerebral microvessel (not drawn to scale). Note the greater pericyte coverage on the retinal endothelium, and the smaller calibre of the retinal vessel. OBF, ocular blood flow; CBF, cerebral blood flow.

Pathological thickening of the retinal microvascular basement membrane occurs notably in diabetic retinopathy (Cai & Boulton, 2002; Tsilibary, 2003), as well as in the rare genetic small vessel disease, cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL) (Haritoglou et al. 2004a).

Surrounding glial cells

Both the cerebral and the retinal microvasculatures are surrounded by numerous astrocytic processes (known as perivascular end feet) (Abbott et al. 1992; Holash & Stewart, 1993). *In vitro* studies show that as well as providing structural support, these astrocytic processes play an important role in the development of endothelial zonulae occludens (ZO-1 expression), and the production by cerebral endothelial cells of specific blood–brain barrier proteins (Arthur et al. 1987; Janzer & Raff, 1987; Maxwell et al. 1987, 1989; Meyer et al. 1991; Rauh et al. 1992; Hurwitz et al. 1993; Janzer, 1993; Sun et al. 1995; Joo, 1996; Hayashi et al. 1997). Retinal astrocytes (and Mueller cells) have also demonstrated induction of barrier properties in vascular endothelial cells (Janzer & Raff, 1987; Gardner et al. 1997; Wolburg & Lippoldt, 2002), via the release of humoral factors [such as glial cell line-derived neurotrophic factor (GDNF), bFGF, TGFbeta] and direct contact (Tao-Cheng et al. 1987; Tontsch & Bauer, 1991; Dehouck et al. 1994; Igarashi et al. 1999; Ramsauer et al. 2002). Both cerebral and retinal astrocytes may also play a role in angiogenesis, inducing endothelial cell and pericyte differentiation. In response to hypoxia, retinal astrocytes (which predominate in the nerve fibre layer) stimulate the release of vascular endothelial growth factor (VEGF), which in turn stimulates the growth of retinal blood vessels across the retinal surface, using the astrocytic processes as a template for angiogenesis (Zhang & Stone, 1997). Mueller cells, which extend radially from the inner limiting membrane of the retina to the external limiting membrane, serve as templates for retinal vascular growth inwards to the inner nuclear layer.

Perivascular microglia are a distinct subset of microglia within the central nervous system (Graeber et al. 1989; Stoll & Jander, 1999). The origin of the perivascular microglia has been shown to be from blood-derived monocytic precursor cells, from which they are regularly replaced (Hickey et al. 1992; Lassman et al. 1993). Both cerebral and retinal microglia have phagocytic properties, phagocytosing cerebral and retinal neurons after injury (Schnitzer, 1989; Thanos, 1991; Mato et al. 1996; Schuetz & Thanos, 2004).

Although the inner retinal and cerebral circulations are morphologically very similar, they can exhibit significantly different responses to various insults, and these differences may explain some of the variation between the two vasculatures in certain pathological processes (Lorenzi et al. 1986; Bradbury et al. 1989; Lightman & Yuen, 1989; Kern & Engerman, 1996; Grammas & Riden, 2003).

Regulation of cerebral and retinal circulation

Both the brain and the retina have control mechanisms in place to allow a constant blood flow and hence delivery of nutrients in the face of a broad range of external factors, such as systemic blood pressure (Robinson et al. 1986). This local process of control (autoregulation) is a property of both the inner retinal and the cerebral circulation (Fig. 3).

The perfusion pressure of the cerebral circulation is related to systemic blood pressure and intracranial pressure by the following relationship:

cerebral blood flow = (mean arterial blood pressure – intracranial pressure)/vascular resistance.

The perfusion pressure of the ocular circulation is related to systemic blood pressure and intraocular pressure by a similar relationship:

ocular blood flow = (mean arterial blood pressure – intraocular pressure)/vascular resistance.

In order for the retinal and cerebral circulations to maintain cerebral blood flow over a range of systemic blood pressures, their vascular resistance has to be altered accordingly. This is mediated by the vascular smooth muscle of the cerebral and retinal arterioles and pericytes (Alm & Bill, 1972; Riva et al. 1981, 1986; Delaey & Van de Voorde, 2000b; Vavilala et al. 2002) (predominantly via changes in vessel diameters). Cerebral blood flow is maintained between approximately 50 and 160 mmHg mean arterial blood pressure (MABP) (Wagner & Traystman, 1985; Paulson et al. 1990). In the case of increased intraocular pressure (IOP), the upper limit for autoregulation is approximately within 40–45 mmHg of the MABP (Grunwald et al. 1988). With

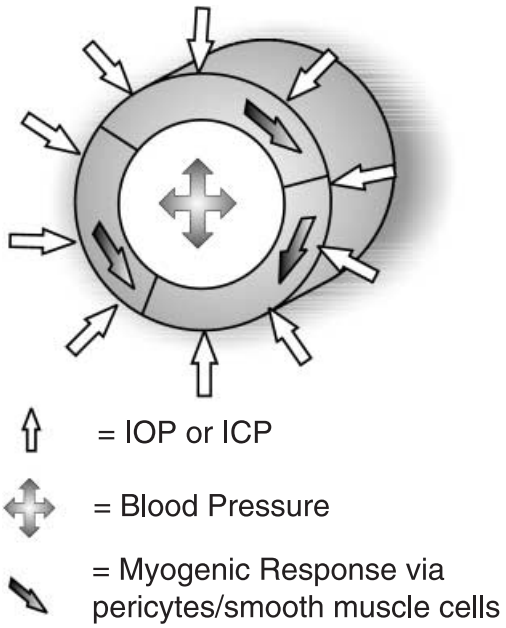

Fig. 3 Schematic diagram of the myogenic (via pericytes/vascular smooth muscle) and metabolic components of vascular autoregulation of the retinal and cerebral microvasculature. IOP, intraocular pressure; ICP, intracranial pressure; NO, nitric oxide.

increases of systemic blood pressure of approximately 40%, autoregulation is overcome, and retinal blood flow will increase (Robinson et al. 1986). The degree of autoregulation within the retinal vascular circulation has been shown to vary, with the region supplied by the superficial capillary bed being better regulated than the deeper capillary bed (Yu et al. 1994), and this may explain the frequent involvement of the deeper capillaries in retinal vascular disease (Yanoff & Fine, 1989).

Autoregulation consists of two components (Fig. 3).

(a) Myogenic

This is defined as the capacity of both vascular smooth muscle cells and pericytes of the retinal and cerebral microvasculature to contract in response to an increase in transmural pressure (Ursino, 1991), and has been directly visualized in isolated retinal and cerebral blood vessels, producing increased vascular tone and decreased luminal diameter (Wallis et al. 1996; Delaey & Van de Voorde, 2000a; Yu et al. 2003).

(b) Metabolic

Cerebral and retinal blood flow is related to local metabolic demand, which depends on regional neuronal activity. Hence, cerebral blood flow is coupled to the regional utilization of glucose (an indicator of neuronal activity) and cerebral oxygen metabolic rate (Hoge et al. 1999; Leenders et al. 1990). In the retina, blood flow has been found to be greater in the temporal retina (containing the metabolically active macula) than in the nasal retina (Riva et al. 1985; Rassam et al. 1996) and increases under conditions of light exposure (Bill & Sperber, 1990a,b; Kondo et al. 1997). Similarly, cerebral regulation of blood flow is driven by metabolic activity (Fox et al. 1988), and can vary regionally within the brain at any one time. As well as the local accumulation of metabolites, local parenchymal and endothelial substances have major impacts on vessel tone, such as nitric oxide (NO) (Palmer et al. 1987; Kondo et al. 1997; Harris et al. 1998), endothelins (Nyborg et al. 1991; Meyer et al. 1993; Dallinger et al. 2000; Polak et al. 2001), arachidonic acid metabolites (Nielsen & Nyborg, 1989, 1990; Hoste & Andries, 1991; Yu et al. 2001) and others (Benedito et al. 1991; Ferrari-Dileo et al. 1991; Ma et al. 1996; Krejcy et al. 1997; Abbott, 2000; Yu et al. 2003).

Neurogenic control of retinal and cerebral blood flow

The retinal vasculature is devoid of autononomic innervation beyond the level of the lamina cribrosa (Ehringer, 1966; Laties, 1967; Ferrari-Dileo et al. 1989; Hoste et al. 1990; Ye et al. 1990), and therefore the regulatory control mechanisms in the retinal circulation are not under neurogenic control and it relies predominantly on local vascular control mechanisms. However, the choroidal circulation is under neurogenic control, and vasoconstriction via sympathetic stimulation (including noradrenergic and neuropeptide-Y fibres) to the

choroidal circulation as well as the extraocular circulation may assist myogenic and metabolic retinal autoregulatory mechanisms (Bill, 1962a,b; Stone et al. 1986). Likewise, there is no autonomic innervation to the cerebral vasculature beyond the pial vessels (Farkas & Luiten, 2001). However, there is evidence of a cholinergic pathway from the basal forebrain to the frontoparietal cortical microvasculature, capable of increasing regional cortical blood flow by vasodilatation (Biesold et al. 1989; Luiten et al. 1996; Barbelieven et al. 1999; Sato et al. 2001; Hamel, 2004), mediated via NO (Adachi et al. 1992; Zhang et al. 1995; Tong & Hamel, 2000). The sympathetic innervation to the large cerebral arteries is derived from the superior cervical ganglion, and includes the neuropeptides norepinephrine and neuropeptide-Y (Uddman & Edvinsson, 1989; Edvinsson et al. 1990). Monoaminergic brainstem centres such as the dorsal raphe nucleus, locus coeruleus or nucleus reticularis pontis oralis also influence vessel tone. The autonomic control of the larger cerebral vessels may exert a degree of regulation of cerebral blood flow, but the finer control on cerebral blood flow is exerted via myogenic and metabolic mechanisms of the cerebral microvasculature.

Evidence of retinal microvascular changes reflecting the cerebral microvasculature in aging and disease

Aging

Both the retinal and the cerebral microvasculatures undergo similar changes with aging. A reduction in cerebral blood flow (Tachibana et al. 1984; T et al. 1986; Marchal et al. 1992; Kawamura et al. 1993; Schultz et al. 1999; Slosman et al. 2001), decreased glucose and oxygen metabolism (Kuhl et al. 1982, 1984; Alavi, 1989; Eberling et al. 1995; Moeller et al. 1996), and impairment of the structural integrity of the anatomy of the microvasculature are all features of the aging brain. Similarly, retinal blood flow decreases incrementally with age (Rimmer et al. 1989; Costello et al. 1992; Grunwald et al. 1993; Williamson et al. 1995; Groh et al. 1996; Embleton et al. 2002), and exhibits decreased metabolic demand (Gramer & Dirmeyer, 1998). A recent study has shown evidence of an age-related decrease in retinal vascular autoregulation, related to increasing systemic blood pressure (Jeppesen et al. 2004). In contrast, another small study observed no such age-related change in retinal vascular diameter response to flicker light (Nagel et al. 2004). The cerebral vasculature undergoes morphological changes with age, including basement membrane thickening, and a decrease in endothelial and pericyte cell populations (Stewart et al. 1987; Mooradian, 1988; de Jong et al. 1991; Mooradian et al. 1991; Luiten et al. 1994; Farkas et al. 2000; Keuker et al. 2000; Farkas & Luiten, 2001). Retinal vascular age-related morphological changes have not been extensively studied. In one study using electron microscopy (Lee et al. 1987), features of aging-related vascular changes in two middle-aged eyes (52 years and 60 years) revealed extensive multilayering of the basement membrane and deposition of collagen. Density of the cerebral microvasculature decreases with age (Abernathy et al. 1993). Between the 6th and 7th decades, normal aged subjects demonstrate increased capillary diameter, volume and total length (Hunziker et al. 1979; Bell & Ball, 1981; Mann et al. 1986).

Retinal vascular changes in cognitive decline and dementia

Vascular dementia accounts for approximately 20% of all causes of dementia (Geldmacher & Whitehouse, 1996; Roman, 2003). Causes of vascular dementia include large cortical–subcortical infarcts and multiple infarcts, subcortical 'small-vessel' disease, and single infarcts in a strategic location critical to mental function (Roman, 2003). It is now recognized that cerebrovascular small-vessel disease with white-matter lesions and lacunar infarcts is an important cause of cognitive impairment of cerebrovascular origin (Erkinjuntti et al. 2000; Inzitari et al. 2000; Jellinger, 2002; Mok et al. 2004). Cerebral white-matter lesions are seen as hyperintensities on T2-weighted MRI scans, typically located in the periventricular areas and the anterior limb of the internal capsule. The severity of white-matter lesions is directly proportional to the degree of stenosis of the medullary arterioles due to arteriosclerosis (van Swieten et al. 1991). Lacunes result from occlusion of the lenticulostriate, thalamo-perforating and long medullary arterioles (Fisher, 1982). Lacunar infarcts are typically located in the thalamus, caudate nucleus, globus pallidus, internal capsule and frontal white matter (Lammie, 2002; Roman et al. 2002). A study in spontaneously hypertensive rats suggests retinal arterial changes may be predictive of cerebral arterial changes in hypertension (Tomassoni et al. 2002). Pathological studies have shown characteristic cerebral arteriolar changes (attenuation, increased

tortuosity, increased capillary microaneurysms) associated with dementia (Miyakawa et al. 1988; Fischer et al. 1990; Kalaria, 1992; Buee et al. 1994; Moody et al. 1997).

Other dementias such as Alzheimer's disease are known to have a vascular component, with small-vessel disease and microinfarction (Ravona-Springer et al. 2003; Thal et al. 2003) and an increasingly recognized cause of dementia in the elderly is mixed Alzheimer's disease/vascular dementia (MRC/CFAS, 2001). As well as being an important cause of dementia in the elderly, cerebrovascular disease is also increasingly recognized as an important factor in development of mild cognitive impairment.

Few studies to date have explored retinal microvascular changes in cognitive impairment (Kwa et al. 2002; Wong et al. 2002b,c). Kwa et al. (2002) found that retinal arteriolar abnormalities, including narrowing, arteriolosclerosis and presence of retinal exudates, correlated with MRI signs of cerebral white-matter lesions. In addition, presence of lacunar infarction correlated with retinal exudation. The authors also found a substantial number of patients with retinal microvascular abnormalities who did not have evidence of cerebral white-matter lesions, and suggest that this may reflect a period of time required before the small vessel changes lead to the development of white-matter lesions. However, their study highlights the problem of using subjective observer-driven techniques to assess retinal microvascular abnormalities, as interobserver agreement was modest. To overcome this, only patients in which consensus was agreed between the two ophthalmologists were included in their study, but this may have an element of bias. However, their study supports the concept of retinal vascular imaging as a useful approach in screening patients at risk of cerebral small-vessel disease, and potentially as an indicator of those patients who may be at increased risk of developing cognitive impairment in later life.

The Atherosclerosis Risk in Communities Study (ARIC) was a large, population-based, cross-sectional study of 15 792 participants, ranging in age from 45 to 64 years. The ARIC study explored the relationship between retinal microvascular abnormalities and cognitive impairment (tested using the delayed word recall test, digit symbol subtest and word fluency test) in this middle-aged population (Wong et al. 2002c) and found that the presence of retinal microvascular abnormalities (presence of any retinopathy, micoraneurysms, retinal haemorrhages and exudates) was independently associated with a small decrease in cognitive function (two standard deviations lower than the mean score). The ARIC study lends further evidence that vascular permeability may be an important element in cerebral vascular changes leading to cognitive decline, as the retinal anomalies most consistently associated with cognitive impairment were micoraneurysms (odds ratio 1.62–3.00) and retinal haemorrhages (odds ratio 1.99–4.10), rather than arteriolar narrowing. Indeed, generalized arteriolar narrowing was not found to be correlated with cognitive function. Microaneurysms and retinal haemorrhages are indicative of more severe microvascular disease (Wong et al. 2002c), and, in conjunction with the finding that the same retinal features are most strongly associated with incident stroke, suggest that blood–brain barrier breakdown may be an important pathological feature in both cognitive impairment and stroke. An important drawback of the ARIC study is that the cognitive function tests were not done contemporaneously with the retinal photography, but were performed either 3 years previously or afterwards, and that the absence of visual acuity measurements may have had an effect on the outcomes, if those who could not optimally perform the cognitive function tests had visual impairment.

In addition, the ARIC study published evidence of retinal microvascular changes occurring independently in association with both MRI-defined white-matter lesions (Wong et al. 2002a) and sulcal widening and ventricular enlargement on MRI in this middle-aged population (Wong et al. 2003c). Again, the more severe signs of microvascular damage (microaneurysms, haemorrhages and exudates) were most strongly associated with MRI-defined white-matter lesions and cerebral atrophy. This study also showed that MRI-defined white-matter lesions were independently related to risk of clinical stroke. In the presence of retinopathy, those with MRI-defined white-matter lesions were 18.1 times more likely to develop stroke than those without either white-matter lesions or retinopathy.

The ARIC study also provided indirect evidence of retinal vascular abnormalities being related to impaired cognitive performance. They found a weak association between early age-related maculopathy (ARM) and cognitive function (word fluency test) in a middle-aged population (Wong et al. 2002b). Both ARM and cognitive function may share common vascular risk factors (Klein et al. 1993, 1997; Vingerling et al. 1995; Ott et al. 1998; Knopman et al. 2001). Indeed, the Blue Mountains

Eye Study found a weak association between retinal microvascular changes (focal arteriolar narrowing and arteriovenous nicking) and ARM progression (Klein et al. 1993; Wang et al. 2004). The Rotterdam Study also found a weak association between late ARM and incidence of Alzheimer's disease in a population over 75 years of age (Klaver et al. 1999).

Retinal morphological changes are known to occur in Alzheimer's disease, including depletion of optic nerve ganglion cells, loss of nerve fibre layer and abnormal pattern ERG responses (reduced implicit time and amplitude) (Hinton et al. 1986; Katz et al. 1989; Trick et al. 1989; Blanks et al. 1996a,b; Parisi et al. 2001). Beta-amyloid and amyloid-associated proteins related to the pathogenesis of Alzheimer's disease have been isolated in retinal ganglion cells and nerve fibres (Loffler et al. 1995). There may be a common vascular component to the neurodegenerative process of both Alzheimer's disease and the resultant neuronal loss both at the cerebral and the retinal level.

There have been some studies looking at the association of diabetic retinopathy as a marker of microangiopathy, and its association with cognitive function. Some of these studies have been confounded by the presence of co-morbidity, such as hypertension (Dejgaard et al. 1991; Yousen et al. 1991; Lunetta et al. 1994). One study found an association between presence of microangiopathy and the presence of leukoaraiosis (Dejgaard et al. 1991), whereas another study found no evidence of cerebral MRI abnormalities in a cohort of patients who had had laser treatment for proliferative diabetic retinopathy (Yousen et al. 1991). More recently, Ferguson et al. (2003) reported on a cross-sectional study of young people with type 1 diabetes and found an association between the presence of diabetic retinopathy and small focal white-matter hyperintensities in the basal ganglia. In addition, presence of background diabetic retinopathy was associated with different domains of cognition, including fluid intelligence, information processing and attention ability. This association persisted when age, gender, premorbid IQ and duration of diabetes were accounted for. A possible explanation for the association may be a difference in visual function affecting the cognitive tests in the patients with background diabetic retinopathy. However, visual function processing, as assessed by the visual evoked potential P100 latency, is unaffected by background retinopathy, suggesting that the most likely explanation is that the retinal diabetic microangiopathy acts as a marker of suboptimal diabetic control, with chronic hyperglycaemia (Parisi et al. 1994). Long-duration type 1 diabetes complicated with retinopathy is known to be associated with impaired cerebrovascular responsiveness (Fulesdi et al. 1997).

Retinal and cerebral microcirculatory changes in hypertension and diabetes

Two of the most important cardiovascular risk factors for stroke are hypertension and diabetes. Both the cerebral and the retinal microcirculations exhibit morphological changes in these two conditions. However, whereas in hypertension the pathological changes are very similar, diabetes demonstrates how the microvasculatures may differ in response to certain conditions.

(a) Hypertension

Both cerebral and retinal microvasculatures undergo morphological changes with increasing blood pressure (Hill, 1970; Goto et al. 1975; Tso & Jampol, 1982). Hypertension causes generalized retinal arteriolar narrowing. In vessels with arteriolosclerosis, focal narrowing and dilatation may occur. The sclerotic phase is associated with tunica media hyperplasia and hyaline degeneration of the arteriolar wall, and in addition to vessel attenuation, may be associated with arteriovenous nicking and arteriolar tortuosity. Continued high blood pressure may lead to exudative changes and blood–retinal barrier breakdown, with fibrinoid necrosis, luminal narrowing and ischaemia, leading to retinal haemorrhages, micoraneurysms, exudates and nerve fibre layer ischaemia (cotton wool spots). Retinal microvascular changes appear not only to reflect current blood pressures, but also past blood pressures (Sharett et al. 1999), in particular generalized arteriolar narrowing and arteriovenous nicking.

Similar cerebral microvascular changes occur in hypertension, including hyaline arteriosclerosis (Lammie, 2002), leading to luminal narrowing (which correlates with systemic blood pressure) (Furuta et al. 1991). Pathologically, tunica media and the internal elastic lamina degenerate and are replaced by fibrous tissue. This leads to increased vessel tortuosity, and increased vessel permeability (Fredriksson et al. 1988), owing to breakdown of the blood–brain barrier. The pathological hallmark of acute hypertensive brain damage is fibrinoid necrosis (Gustafsson, 1997).

(b) Diabetes

The retinal microvascular changes occurring in diabetes mellitus are well documented (Cai & Boulton, 2002), including loss of retinal pericytes, basement membrane thickening, capillary microaneurysm formation, increased vascular permeability leading to exudation and tissue oedema, and capillary occlusion causing ischaemia, which may lead to retinal neovascularization. The cerebral circulation shows some significant differences and is much less affected in diabetes (Kern & Engerman, 1996). Cerebral vascular lesions reported to occur in diabetes are predominantly capillary basement membrane thickening (Mukai et al. 1980; Johnson et al. 1982; Frank et al. 1987a) or alterations in number or tortuosity of capillaries (Mukai et al. 1980; Jaboksen et al. 1987). Studies have found no evidence of pericyte loss or damage in histological cross-sections of cerebral cortex from patients said to have diabetic retinopathy (de Oliveira, 1966; Addison et al. 1970), and concluded that pericyte loss developed preferentially in the retina. Kern & Engerman (1996) found that microaneurysms, acellular capillaries and pericyte 'ghosts' developed in retinas of dogs with diabetes or galactosaemia, but did not develop in cerebral cortical vessels from the same animals. As stated earlier, pericytes cover more of the capillary circumference in retina than in brain (Frank et al. 1987b, 1990). Greater loss of pericytes from the retina compared with the cerebral cortex in diabetes thus might lead to differences in blood flow regulation between the two tissues. Consistent with this, Kern & Engerman (1996) found that capillary diameter tended to be increased in retinal capillaries from animals with diabetes, but cerebral capillaries were not. There are significant differences in the rate of glucose uptake between retinal and cerebral endothelial cells (Badr et al. 2000; Tang et al. 2000; Rajah et al. 2001), although the results are somewhat contradictory. Whereas Rajah et al. (2001) found an inability of retinal endothelial cells to down-regulate glucose uptake in the presence of high glucose levels, suggesting that this could make retinal microvessels more sensitive to the deleterious effects of hyperglycaemia, Badr et al. (2000) did find a down-regulation of the specific carrier-mediated transport protein GLUT-1 in retinal endothelial cells, but that no such down-regulation occurred in the cerebral microvasculature. Tang et al. (2000) reported a decrease in GLUT-1 expression in both tissues (55 vs. 36%, respectively). In addition, they report an increase in retinal endothelial glucose concentration in diabetes, with no such increase noted in cerebral tissue. Further studies are needed to clarify exactly the differences in glucose uptake between these two microvasculatures in hyperglycaemia. Other biochemical disparities include differences in gamma-glutamyl transpeptidase (Kowluru et al. 1994), protein kinase C (Shiba et al. 1993) and rate of glucose oxidation (Kennedy et al. 1983). One of the effects of diabetes on the retinal circulation is a reduction and redistribution of occludin in retinal endothelial cells with resultant increased vascular permeability (Antonetti et al. 1998; Barber et al. 2000). ZO-1 expression is greatly increased in brain-derived endothelial cells under hyperglycaemia, whereas retinal endothelial cells are unaffected (Grammas & Riden, 2003). Thus the cerebrum shows little or no permeability defect in diabetes (Mooradian, 1997). There is evidence to suggest that the retina has a reduced response to oxidative stress compared with the cerebral circulation with relatively lower levels of glutathione peroxidase and superoxide dismutase (Grammas & Riden, 2003).

Evidence of retinal microvascular changes in stroke

There is evidence that retinal microvascular anomalies reflect cerebrovascular changes relating to stroke (Aoki, 1975; Okada et al. 1976; Svardsudd et al. 1978; Tanaka et al. 1985; Sano et al. 1994; Kobayashi et al. 1997). After adjusting for other risk factors (age, sex, race, blood pressure, diabetes), the ARIC study found retinal microvascular anomalies were predictive of incident stroke (including ischaemic stroke) as well as MRI-detected subclinical stroke (Wong et al. 2001a). The relationship between retinal microvascular changes and stroke was strongest for micoraneurysms and soft exudates (adjusted relative risk 3.11 and 3.08, respectively).

The Cardiovascular Health Study similarly found a relationship between retinal microvascular changes and stroke, after controlling for blood pressure and other risk factors (Wong et al. 2003b). Participants with retinopathy were twice as likely to have a stroke as those without retinopathy (odds ratio 2.0, 95% CI 1.1–3.6).

The Beaver Dam Eye Study found an association with the presence of retinal microaneurysms, haemorrhages, and retinal arteriolar narrowing and 10 years risk of stroke and coronary heart disease mortality (Wong et al. 2003a).

In a prospective study, the Blue Mountains Study found an increased relative risk for all forms of

retinopathy (Wong, 2004), and unlike previous studies, the association with generalized arteriolar narrowing was as strong as the association with focal microaneurysms and haemorrhages [relative risk 3.0 (95% CI 1.1–8.2) vs. 3.0 (95% CI 1.9–5.2), respectively].

Other investigators have found a relationship between lacunar infarcts and retinal microvascular abnormalities (Korber et al. 1986; Schneider et al. 1993), and autopsy studies have shown a correlation between retinal and cerebral vasculature changes in patients who had died of stroke (Goto et al. 1975). Studies have also shown retinal arteriolar changes in the spontaneously hypertensive rat (Hamada, 1993). In addition, Kappelle et al. (1988) found in a prospective study that 78% of patients with lacunar stroke had retinal microvascular abnormalities. In another study, there was no difference in the prevalence of retinal lesions in patients with either lacunar infarcts or cortical strokes (Luijckx et al. 1998). Interestingly, Hiroki et al. (2003) reported on the association between central retinal artery Doppler parameters and cerebral small-vessel disease. They found that end-diastolic and mean velocities was related to the severity of cerebral small-vessel disease, independently of aging.

Hereditary small-vessel disease

This forms a small but important group of conditions that serve to illustrate the close relationship between the two barrier microcirculations of the brain and the retina, and their similar morphological response to disease. They include the conditions CADASIL, cerebrovascular retinopathy (CRV), hereditary endotheliopathy with retinopathy, nephropathy and stroke (HERNS) and hereditary vascular retinopathy (HVR).

CADASIL

CADASIL is a rare autosomal dominant microangiopathy, resulting from defects in the *NOTCH 3* gene of 19q13.1 (Joutel et al. 1996), encoding for a receptor expressed on vascular smooth muscle cells and pericytes (Joutel et al. 2000). The clinical characteristics of those affected include recurrent transient ischaemic attacks (TIAs), early onset stroke, dementia and migraine with auras (Nishio et al. 1997; Dichgans et al. 1998; Haritoglou et al. 2004a,b). Patients invariably have diffuse white-matter hyperintensities and lacunar infarcts on neuroimaging (Dichgans et al. 1998). Retinal vascular findings are well documented in CADASIL, and have been found to resemble closely those of the cerebral microcirculation. Retinal features described include retinal arteriolar sheathing and attenuation (Robinson et al. 2001; Haritoglou et al. 2004b), arteriovenous nicking and remnants of branch retinal vein occlusions (Haritoglou et al. 2004b). Histopathological examinations of cerebral medullary arteries have shown loss of vascular smooth muscle cells, with adventitial fibrosis, but no evidence of vascular occlusion (Ruchoux et al. 1995; Ruchoux & Maurage, 1997; Okeda et al. 2002). Histopathological examination of retinal arterioles also shows loss of vascular smooth muscle cells, perivascular fibrosis, with thickened basement membrane and granular osmiophilic material in arterial walls, but with no evidence of arteriolar occlusion (Haritoglou et al. 2004a). Interestingly, no evidence of any choroidal vascular abnormalities was detected. The pathological hallmark of CADASIL (deposition of granular osmiophilic material within the basement membrane of smooth muscle cells) can be detected in other tissues outside of the central nervous system, and indeed skin or muscle biopsy is the normal mode of diagnosis of CADASIL. However, the clinical manifestations seem to be restricted to the cerebral and retinal microcirculations, possibly reflecting their specialized barrier function.

Cerebrovascular retinopathy

CVR is a rare genetic condition characterized by abnormal vasculature in the brain and retina (Grand et al. 1988; Gutmann et al. 1989). Unlike CADASIL, CRV is characterized by retinal capillary occlusion, with fluorescein angiography showing capillary closure, and the presence of shunt vessels. Histopathological examination of brain lesions (which often form intracerebral masses mimicking a brain tumour on neuroimaging: Weil et al. 1999; Niedermayer et al. 2000a,b) reveal arterial wall thickening, perivascular fibrosis, thrombosis and occlusion of small vessels, with fibrinoid necrosis and adjacent marked oedema and astrogliosis.

HERNS

HERNS was first described by Jen et al. (1997), affecting 11 members of a Chinese American family over three generations in an autosomal dominant inheritance pattern. Retinal changes described include retinal capillary occlusion, perifoveal telangiectasias and macular

oedema. Neurological features included migraine, dysarthria, hemiparesis and apraxia. Neuroimaging revealed multiple subcortical lesions with surrounding oedema. In addition to brain and retinal involvement, several family members had renal involvement with proteinuria and haematuria. Brain biopsy from one of the patients revealed occlusion of small blood vessels, with intense subcortical white matter astrocytic gliosis. HERNS may be part of the same spectrum as CRV, in which systemic involvement has not been documented.

Hereditary vascular retinopathy

HRV is another autosomal dominant condition characterized by retinal occlusive microangiopathy (Storimans et al. 1991). As well as retinal features, cerebral symptoms, including migraine, mild cognitive decline and depression, have been described. White-matter abnormalities have also been reported.

HVR, CRV and HERNS have all been mapped via linkage analysis to a single locus on chromosome 3p21.1–p21.3 (Ophoff et al. 2001). Recently, a new hereditary small-vessel disease of the retina and brain has been described (Dichgans, 2003; Vahedi et al. 2003), characterized by retinal arterial tortuosity and retinal haemorrhages. Cerebral manifestations include infantile hemiparesis and migraine with aura. Neuroimaging revealed diffuse leucoencephalopathy with dilated perivascular spaces. Genetic analysis revealed no linkage to the 3p21 locus. This may be related to the condition hereditary retinal arterial tortuosity with retinal haemorrhages (Goldberg et al. 1972; Clearkin et al. 1986), which was believed only to affect the retinal arteries, although there is one report of a case with this condition suffering migraine, and having multiple small areas of midbrain ischaemia on neuroimaging (Sears et al. 1998).

These rare inherited small-vessel diseases typically involve both the inner retinal and the cerebral circulations. They illustrate how the pathological event occurring in the cerebral circulation seems to be mirrored by the retinal circulation. In any patient presenting with evidence of small-vessel disease, ophthalmoscopy is recommended to help in making the diagnosis (Robinson et al. 2001; Dichgans, 2003), particularly in young patients (Vahedi et al. 2003).

Table 1 summarizes the main associations found between retinal microvascular changes and stroke, cognitive impairment, cerebral white-matter lesions and cerebral atrophy.

Retinal vascular image analysis and its potential role in predicting cerebrovascular changes

Subjective evaluation of the retinal vasculature has been found to be unreliable (Kagan et al. 1966; Aoki, 1975; Dimmitt et al. 1989). More objective methods to assess the retinal microvascular topography are now possible, owing to the development of digital retinal image processing techniques. Digitalized image analysis techniques have been shown to be more reliable than previous micrometric techniques (Delori et al. 1988; Newsom et al. 1992; Sherry et al. 2002).

Table 1 Associations (odds ratios +95% confidence intervals) between cerebrovascular diseases and retinal microvascular changes

	Generalized arteriolar narrowing	Focal arteriolar narrowing	Focal retinopathy (microaneurysms, hamorrhages, exudates, cottonwool spots)
Cognitive impairment (ARIC)	1.1 (0.8–1.5)	0.6 (0.4–0.9)	2.6 (1.7–4.0)
Stroke			
(a) CHS	1.1 (0.7–1.8)	1.2 (0.6–2.4)	2.0 (1.1–3.6)
(b) ARIC†	1.2 (0.7–2.3)	1.2 (0.7–1.9)	2.6 (1.6–4.2)
(c) BMES	3.0 (1.1–8.2)	2.6 (1.5–4.4)	3.0 (1.9–5.2)
Cerebral white-matter lesions			
(a) ARIC	1.2 (0.8–1.9)	2.1 (1.4–3.1)	2.5 (1.5–4.0)
(b) Kwa et al.	2.3 (1.1–4.6)*	Not assessed	3.4 (1.5–8.1)
Cerebral atrophy (ARIC)	1.0 (0.7–1.4)	1.1 (0.8–1.6)	1.9 (1.2–3.0)

ARIC, Atherosclerosis Risk in Communities Study; CHS = Cardiovascular Health Study; BMES = Blue Mountains Eye Study.
*Generalized narrowing subjectively assessed by ophthalmologist.
†Prospective study; relative risk data.

Image acquisition

Photography is the most common technique used to acquire retinal vascular images. This has usually been conventional film photography, using a fundus camera and requiring separate film processing. Processed photographic images can then be digitalized before being subjected to image analysis. However, recent improvements in the resolution of direct digital cameras has allowed direct digital capture of images using a charged coupled device (CCD) directly attached to a fundus camera. This has the advantage of immediate image acquisition, without the need for film processing. It also enables one to assess instantly the quality of the image, and repeat the process if necessary, in order to optimize the quality of the image. Any poor-quality images can be instantly discarded.

As well as conventional and digital photography, other newer techniques have been developed for retinal image acquisition. The retinal vessel analyser (RVA®; Imedos, Weimar, Germany) consists of a retinal fundus camera, a CCD video camera, a real-time monitor for electronic online image acquisition, and a PC for overall system control, image analysis and result archiving (Seifert & Vilser, 2002; Vilser et al. 2002). It allows real-time assessment of retinal vascular diameters at a maximum frequency of 50 Hz (allowing 25 vessel diameter readings per second) and has demonstrated reproducible results (Polak et al. 2000; Pache et al. 2002). Adaptive algorithms allow for measurement of retinal vessel widths, utilizing the absorbing properties of haemoglobin in each blood vessel. The system is able to correct automatically for slight adjustments in luminance that may occur due to slight eye movement, and thus vessel diameter can be recorded as a function of time as well as position along the vessel. A major limitation of the RVA® is that it assumes that the eye under measurement has no refractive error (emmetropia) and uses standardized units to measure vessel diameters. Therefore, the RVA® is unable to give actual measurements of vessel wall widths if a significant number of subjects may not conform to the assumptions of emmetropia. However, attempts at finding a value for the diameter of the central retinal artery *in vivo* using the RVA® have been performed, utilizing the diameters of all retinal veins entering the optic disc and laser Doppler velocimetry as a measure of the total retinal blood flow, and combining this with the velocity of blood flow in the central retinal artery (Dorner et al. 2002).

The scanning laser ophthalmoscope (SLO) (Nagel et al. 1992) provides a high-quality image of the fundus using less than 1 : 1000 of the light necessary to illuminate the fundus with conventional light ophthalmoscopy. During image acquisition, only one point on the fundus is illuminated at any one time. The laser sweeps across the fundus in a raster-like fashion so that a piece-by-piece image of the fundus is built up on the monitor. In addition, because the SLO only illuminates a small area of the fundus at any one time, only a small amount of the patient's pupil is used for illumination. This means that pupil dilation is not usually necessary when acquiring fundal images with the SLO. However, the optical resolution of the SLO is currently only 10–20 μm per pixel, and therefore is currently insufficient to be able to produce accurate measurements of retinal vessels. A non-mydriatic wide-field SLO is now commercially available.

The SLO can be combined with laser Doppler flowmetry to obtain measurements of retinal blood vessel diameter. A tracking stripe provided by a green 543-nm HeNe laser orientated perpendicular to the target vessel is used to measure the retinal vessel. The diameter of the vessel is determined automatically by computer analysis of the signal produced by the image of the vessel on the CCD sensor using the half height method described below. In order to correct for the refractive status of the subject, the operator has to input the axial length of the eye, and the Laser Doppler Flowmeter automatically measures the refractive error of the eye. A distinct advantage of the laser Doppler flowmeter in measuring individual vessels is that it takes numerous measurements over a 2-s time period, and therefore can be averaged to account for the different stages of the cardiac cycle. However, a significant drawback of the technique is that the resolution is limited to vessels greater than approximately 60 μm in diameter.

As well as measuring retinal blood vessel diameter, the laser Doppler flowmeter is able to calculate retinal blood flow, volume and velocity. Light reflected or scattered at moving objects is frequency shifted due to the optical Doppler effect. It interferes with unchanged light reflected at surrounding tissue. The interference causes characteristic temporal intensity variations of the measurable reflected light intensity. This frequency shift can then be used to obtain information on retinal blood flow, volume and velocity. However, the measurements obtained from laser Doppler flowmetry shows variable reproducibility (Guan et al. 2003; Yoshida et al. 2003; Jonescu-Cuypers et al. 2004).

Image analysis

Measurement of retinal vessel widths

One of the most important topographical features of retinal vessels amenable to objective measurement is the retinal vessel wall widths. Attempts at quantifying retinal arteriolar calibres were first considered by Wagener et al. (1947). During the 1960s and 1970s, the introduction of retinal photography allowed semi-objective methods of performing measurements on retinal vasculature using enlarged projected images (micrometric methods) and calipers (Burgess, 1967; Hodge et al. 1969; Parr & Spears, 1974a,b; Cunha-Vaz & Lima, 1978; Bracher et al. 1979; Arzabe et al. 1990; Hubbard et al. 1992). Since the mid-1980s, the introduction of digital image analysis has been used to provide more objective measurements of retinal vascular parameters, including measurements of vessel calibre (Brinchmann-Hansen, 1986; Delori et al. 1988; Eaton & Hatchell, 1988; Newsom et al. 1992; Penn & Gay, 1992; Rassam et al. 1994; Stromland et al. 1995; Wu et al. 1995; Gao et al. 2000).

These techniques commonly rely on intensity profiles of a greyscale image of the fundus (densitometry). A standard greyscale image is produced by eliminating the hue and saturation in the digitized colour fundus photograph while retaining the luminance. The subsequent intensity values will have a range from 0 (black) to 255 (white). A greyscale image consists of many elements, or pixels. The location of each pixel can be identified with spatial coordinates and each has a defined intensity, known also as its grey value.

An intensity profile of a line crossing perpendicular to the blood vessel will tend to produce a distinct double-Gaussian curve against the background intensity of the surrounding retina (Figs 4 and 5). The single Gaussian model is given by the equation:

$$f(x) = a_1 e - \left(\frac{x - a_2}{a_3}\right)^2 + a_4$$

where a_1 is the the amplitude of the peak of the profile, a_2 is the the position of the peak, a_3 is a specific variable of the Gaussian function that controls the width of profile and a_4 is the the background retinal intensity.

A smaller central Gaussian function (representing the central light reflex from retinal arterioles) is subtracted from this model, to produce the double-Gaussian curve. This central reflex is often absent in older eyes. The double-Gaussian curve can then be analysed using image processing software, and an estimate of the width of the blood vessel can then be obtained. The most common technique for acquiring the vessel width is to estimate the width of the vessel at half the height of the peak of the intensity profile of the double-Gaussian curve (half-height method). This strategy minimizes any effect of defocusing at the point of image acquisition, which otherwise may have an effect on the vessel width measurement.

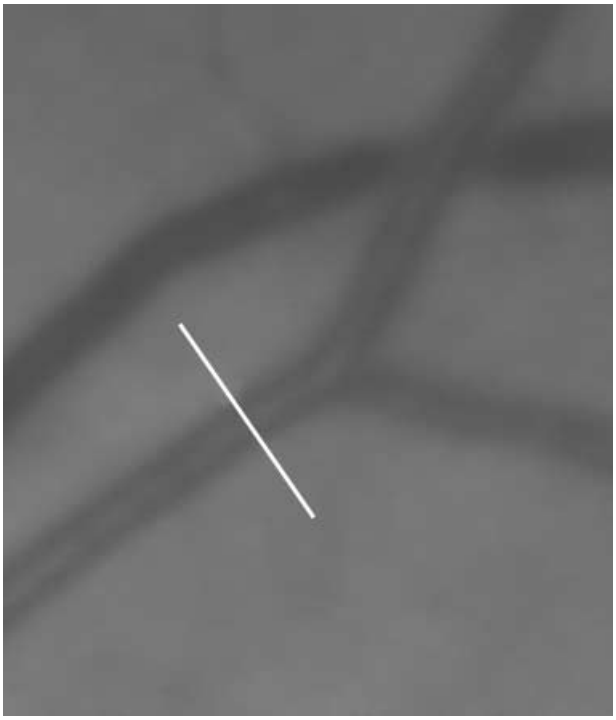

Fig. 4 A greyscale image of a region of the retina, with a line drawn perpendicular to a retinal arteriole. Note the central light reflex from the arteriole.

Other techniques of automated vessel width measurement have included the use of edge detection masks (Gonzalez & Woods, 1992) and sliding linear regression filters (Chapman et al. 2001; Gang et al. 2002). Digitalized image analysis techniques have been shown to be more reliable than previous micrometric techniques (Delori et al. 1988; Newsom et al. 1992; Sherry et al. 2002).

However, all images captured from the retina are subject to image magnification, depending on the distance from the camera to the eye, and also the refractive error of the eye (Pach et al. 1989; Arnold et al. 1993). Therefore, the measurements recorded from any particular individual cannot be directly compared with another individual. Hence, the use of dimensionless

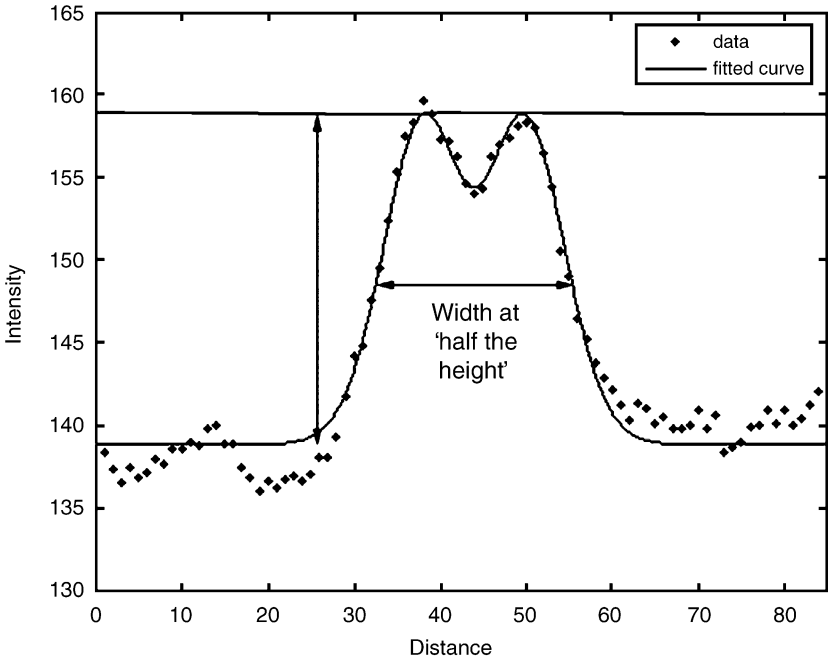

Fig. 5 A typical intensity profile of a cross-section of a retinal arteriole. A curve of best fit has been superimposed on the actual intensity data, showing a double-Gaussian configuration. The height of the intensity profile is calculated by subtracting the background intensity from the peak intensity measured across the vessel. The width of the blood vessel is then measured at the 'half-height'.

measurements has been used to overcome this problem, so that different images from different subjects can be compared. The most commonly performed dimensionless measurement that has been used as a measure of the width of the retinal vessels is known as the arteriolar–venular ratio (AVR) (Hubbard et al. 1992, 1999; Stanton et al. 1995a). Hubbard et al. (1992) extended a previously published method for evaluating an index of generalized arteriolar width [central retinal artery equivalent (CRAE)] developed by Parr & Spears (1974a). The extended technique (known as the Parr–Hubbard formula) produced a method to evaluate an index of generalized venular width (central retinal vein equivalent, CRVE), and then combined this with the central retinal artery equivalent to obtain a generalized AVR. The AVR has other advantages, in that it takes into account the wide variety of vessel calibre within the normal population and the fact that people with narrow arterioles tend to have narrow venules (Wong et al. 2001b). Another dimensionless measure, which is predominantly affected by changes in vascular calibre, is the length–diameter ratio (L:D) (King et al. 1996). This is calculated as the length from the midpoint of a particular vascular bifurcation to the midpoint of the preceding bifurcation, expressed as a ratio to the diameter of the parent vessel at the bifurcation. Changes in the L:D should reflect changes in the widths of the vessels, rather than the length. The L:D has been found to be increased in hypertension (King et al. 1996). No relationship was observed between L:D and presence of peripheral vascular disease (Chapman et al. 2002). No studies have explored a relationship between L:D and cognitive function or stroke.

In addition to image magnification, any defocusing of the image will also have an effect on the measurements obtained from the final image (Heier & Brinchmann-Hansen, 1989). Although using the half-height method to measure vessel widths will minimize any effect of defocusing, it is important to obtain as sharp an image as possible. This is not always possible when photographing patients with medial opacities such as cataracts. Poor-quality captured images can be enhanced using grey-level transformation functions to modify the intensities (Gonzalez & Woods, 2002). This will improve the contrast of the retinal vessels, making the analysis easier. The simplest enhancement technique is a linear contrast stretch that linearly maps values in the input intensity image to new values in the output image to increase contrast. A slightly more complicated approach is histogram equalization, which involves transforming the intensity values of the input image so that the histogram of the output image approximately matches a specified histogram. The resulting output image contains intensity values that are nearly uniformly distributed throughout the range from 0 to 255. Alternatively, contrast-limited adaptive histogram equalization

Fig. 6 (a) An unenhanced RGB image showing moderate delineation of the blood vessels. (b) Image in (a) after greyscale conversion and CLAHE enhancement. The retinal vessels are clearly more prominent.

(CLAHE) can be used (Fig. 6a,b). This method operates on small regions in the image by enhancing the contrast so that the histogram of each output region approximately matches a specified histogram. The overall effect is to highlight subtle detail that would have been lost under normal histogram equalization. Fluorescein angiography can be used to enhance vessel contrast, but has the limitation of being an invasive procedure associated with occasional severe reactions.

Reliability of retinal vessel width measurements

Because of the scale and size of retinal blood vessels, vessel measurements (often approximately 10–20 pixels in diameter) need to be accurate and reproducible. Newsom et al. (1992) compared the retinal vessel width measurement techniques of observer-driven micrometric techniques (making manual measurements from a projected image) and objective computer-driven

microdensitometry, based on a vessel's profile 'grey-level' intensity level, using the previously described 'half-height' technique, which has been shown to be the most accurate in the presence of focusing errors (Brinchmann-Hansen, 1986). The coefficient of variation for computer-driven microdensitometry was calculated as 1.5–7.5%, compared with 6–34% for the observer-driven technique. In addition, there was a tendency of the observer-driven technique to underestimate the size of small vessels. Other studies have shown a coefficient of variation of 1.5% using microdensitometry (Brinchmann-Hansen, 1986) Delori et al. (1988) also found a greater variability for micrometric than microdensitometric techniques.

In the ARIC study (Hubbard et al. 1999), correlation analysis (R) was used to evaluate interobserver agreement (R = 0.74, 0.77 and 0.79, for CRAE, CRVE and AVR, respectively, n = 151 eyes). For intraobserver agreement, R = 0.69, 0.89 and 0.84 for CRAE, CRVE and AVR, respectively.

Sherry et al. (2002) assessed the intra- and interobserver (two observers) reliability (using weighted kappa and correlation statistics) of computer-assisted retinal vessel measurement in the Blue Mountains Eye Study, using a similar system to that used in the ARIC study. They report intraobserver reliability kappa values ranging of 0.8 (for trunk AVR ratios) to 0.93 (for CRVE measurements). R^2 correlation analysis showed agreement ranging from R^2 = 0.79 to 0.92. For interobserver reliability, kappa ranged from 0.71 (for branch AVR measurements) to 0.9 (for CRVE measurements), and correlation statistics showed R^2 ranging from 0.78 to 0.9. Although it is unclear as to why they chose a kappa statistic normally reserved for the analysis of agreement for categorical data, and correlation analysis is clearly vulnerable to high values in the presence of systematic bias (Ludbrook, 2002), these values suggest a reasonable level of agreement using computer-assisted vessel measurement. They also show better agreement for larger vessels (CRVE) and better intraobserver than interobserver agreement. For focal retinopathy (e.g. micoraneurysms and haemorrhages), kappa values range from 0.8 to 0.99 (Couper et al. 2002; Sherry et al. 2002; Wong et al. 2003a).

Other potential problems with image analysis include the fact that retinal vessel widths vary according to the cardiac cycle (Chen et al. 1994; Dumskyj et al. 1996; Knudtson et al. 2004), degree of systemic autonomic nerve stimulation (Lanigan et al. 1988) and degree of fundus pigmentation (Hubbard et al. 1992). Because retinal arterioles are small (approximately 50–200 μm in width), very high-resolution digital images must be obtained to perform accurate measurements from vessels that may be as small as 15–20 pixels in width.

In any study attempting to quantify changes in retinal vascular calibres, other factors that may affect the retinal microcirculation such as age, sex (Wellman et al. 1996; Sharett et al. 1999), hypertension (Sharett et al. 1999; Nagel et al. 2004; Wong et al. 2004a,b), cigarette smoking, diabetes (Klein et al. 2004), intraocular pressure (Nagel et al. 2000), and use of systemic medications and drugs (Tamaki et al. 1996; Okuno et al. 2002; Huemer et al. 2003; Polak et al. 2003) may need to be controlled.

Geometrical measurements at retinal vascular branching points

The geometric angle subtended between two branching offspring blood vessels at a bifurcation junction is an important aspect of arterial network topography (Murray, 1926b). Studies using digital image analysis techniques (Fig. 7) have shown that this angle between two offspring retinal arterioles is reduced in hypertension (Stanton et al. 1995) and in men with low birth weight (Chapman et al. 1997), but another study found no association between the angle at vascular bifurcations and peripheral vascular disease (Chapman et al. 2002). No studies to date have explored the relationship between retinal vascular bifurcation angles and cognitive function or stroke.

Junctional exponents at retinal vascular junctions

Both theoretical and experimental studies have suggested that arterial diameters at any bifurcation should conform to a relationship that minimizes shear stress throughout any vascular network (Murray, 1926a,b; Zamir, 1976; Sherman, 1981; Zamir & Medeiros, 1982; Woldenberg, 1986). This relationship has been described by the following mathematical relationship:

$$D_0^X = D_1^X + D_2^X$$

where D_0 is the diameter of the parent vessel, D_1 and D_2 are the diameters of the offspring vessels, X is a junctional exponent.

The junctional exponent has been calculated to be approximately equal to X = 3 when vascular network

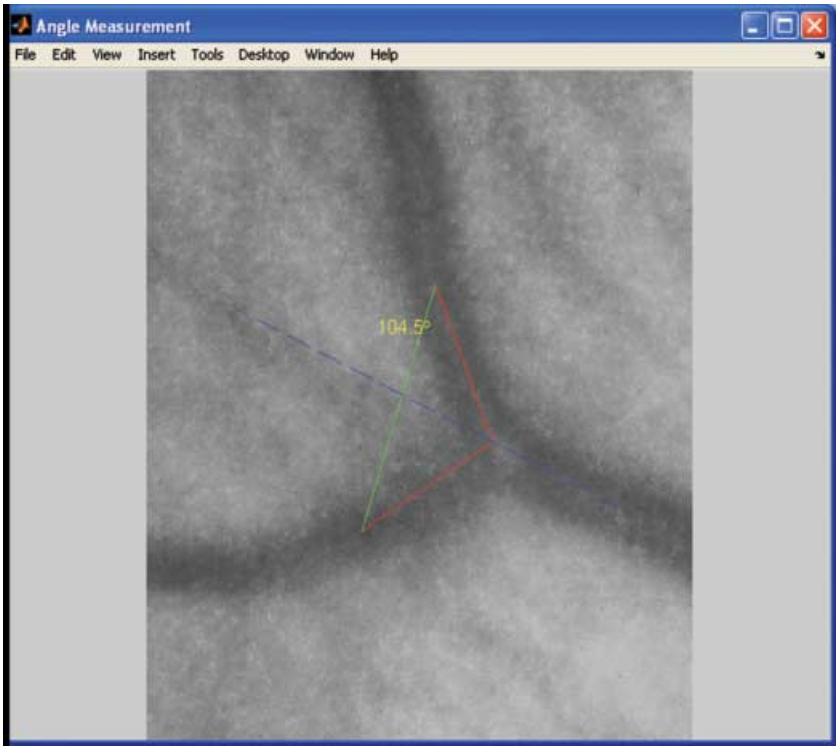

Fig. 7 A region of interest within an enhanced greyscale fundal image has been selected to include a bifurcation of a parent vessel into two daughter vessels. Image analysis programming allows the angle between the two daughter vessels to be calculated.

costs are minimized. Human and animal studies have shown values very close to this ideal value for the retinal circulation (Zamir et al. 1979; Zamir & Medeiros, 1982), as well as the coronary circulation (Hutchins et al. 1976). However, studies have shown that retinal junctional exponents deviate from optimal values with advancing age (Stanton et al. 1995), and in association with peripheral vascular disease (Chapman et al. 2002).

Automated vessel tracking techniques

Vessel tracking describes the process of automated vessel centre localization over a cross-section of a vessel's longitudinal axis, having been given a starting and ending position from which to search (Fig. 8) (Munch et al. 1995; Sinthanayothin et al. 1999). Typically, this takes the form of a Gaussian elongated filter, which is rotated through a number of angles and convolved with the image (Chaudhuri et al. 1989; Thackray & Nelson, 1993; Hoover et al. 2000). By providing information over different sections of the vessel's length, it potentially will give information regarding the variation of vessel widths across its length. This may allow an automated computer-driven objective index of vessel features such as venous beading (Gregson et al. 1995). Vessel tracking may also provide an index of venous tortuosity, by tracking the circuitous route of the blood vessel, and expressing it as a ratio to the shortest length between a nominated starting and end point of the vessel. In addition, automatic identification of retinal structures such as blood vessels (a process known as segmentation) may allow exploration of the fractal

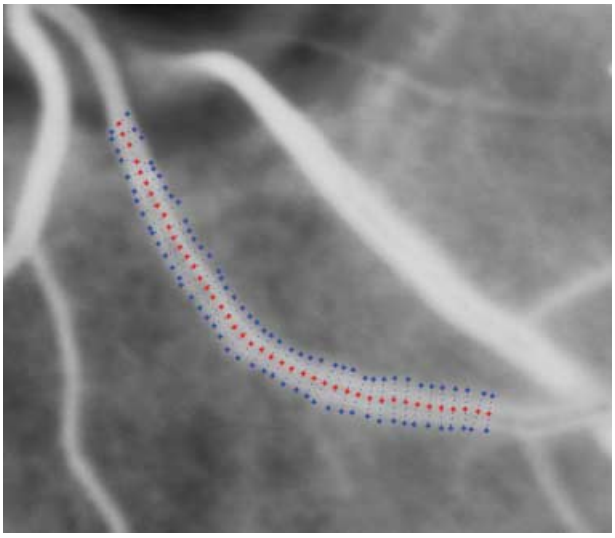

Fig. 8 Greyscale image showing a retinal vessel tracking procedure using a Gaussian elongated filter, having been given starting and ending points.

properties of the retinal vasculature. A study by Mainster (1990) found both retinal arterioles and venules exhibited fractal properties, and suggests that fractal geometry may offer a more accurate description of retinal vascular topography than conventional geometry.

Whilst retinal digital image analysis may offer objective, semi-automated techniques to evaluate topographical features such as retinal vessel calibre, geometry at vessel bifurcations, fractal properties of the vascular network and indices of venous tortuosity, often clinical subjective evaluation of the images is still required to detect focal microvascular abnormalities such as presence of focal arteriolar attenuation, microvascular exudates and retinal haemorrhages. The reliability of grading of focal arteriolar changes has been shown to be largely dependent on the grading system used (Boehm et al. 2002). With the rapid advancement in digital image analysis, it may be possible in the future to enable image processing to detect and quantify these focal abnormalities, using techniques such as segmentation.

Conclusions

In this review, we have outlined the homology that exists between the retinal and the cerebral microcirculations. We have reviewed current evidence that retinal microvascular changes reflect cerebral microvascular changes in aging, as well as in diseases such as vascular dementia and stroke. This forms the basis for investigating retinal vascular topographical features as a potential marker of the state of the cerebral microvasculature in cerebrovascular disease. There are limitations to the sole use of traditional risk factors such as hypertension when assessing an individual's risk for cerebrovascular disease (Prospective Studies Collaboration, 1995), as a substantial proportion of cerebrovascular morbidity is not explained by these risk factors (Khaw et al. 1984; Curb et al. 1996; Menotti et al. 1996). Rather than obtaining a 'snapshot' of current blood pressure at any one particular time, retinal microvascular changes may reflect cumulative microcirculatory changes (Wong et al. 2001b). Current evidence suggests that retinal microvascular changes are independently related to past blood pressure and risk of stroke (Wong et al. 2001b). However, in the case of cognitive impairment, stroke, presence of cerebral white-matter lesions and cerebral atrophy, the strongest retinal microvascular associations (micoraneurysms and retinal haemorrhages) are of relatively low prevalence in the general population (approximately 2–15%; Yu et al. 1998; Wong et al. 2003a,b), and more commonly found lesions (such as generalized arteriolar narrowing) are only substantial in populations with hypertension or diabetes. A recent study found the prevalence of retinal vascular changes in never-treated hypertensives to be much greater than that of other quantitative markers of target organ damage, such as left ventricular hypertrophy, carotid abnormalities and microalbimunria (Cuspidi et al. 2004) and thus retinal vascular changes (based on observer-driven evaluation) cannot be used in amending the cardiovascular risk stratification in this particular population. Thus it is not clear how much more useful information regarding future risk of these clinical entities, over and above current standardized methods, will be obtained by retinal vascular analysis, and future studies need to address this question. As Wong (2004) has pointed out, a consistent demonstration that detection of retinal microvascular changes have independent predictive value and that they substantially improve traditional screening methods for cerebrovascular risk prediction has not been conclusively proved.

The ability to assess the retinal circulation *in vivo* offers potential advantages over other cerebral imaging techniques, which tend to be expensive and not necessarily widely available. Digital retinal image analysis is currently largely a research tool, as it is time-consuming and still undergoing development and refinement. However, digital image analysis of the retinal microvasculature is becoming increasingly available and easier to perform. With improving technology leading to a greater degree of automation (and hence less observer input and less time consumption) and the development of real-time image analysis systems, it may be possible in the future to analyse a digital image of the retinal vasculature quickly, and obtain readily accessible information regarding an individual's potential risk of cerebrovascular disease. Thus it may be possible for retinal vascular analysis to be translated into clinical practice. With the demographic increase in the aged population, the need for earlier identification of patients at risk of cognitive decline and stroke will only become more important, with the potential for earlier therapeutic intervention. Further studies assessing retinal vascular topography changes in cerebrovascular conditions and the continuing rapid advancement in retinal digital image analysis systems may ultimately offer retinal screening as a useful tool in risk identification for cerebrovascular disease.

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