Endothelial (DYS)Function: Quo vadis, Cur vadis

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Endothelial dysfunction is surely one of the greatest problems in cardiovascular disease today. However importance stretches well beyond the area of major interest, and the conventional tests, whose value in clinical trials, and whose reproducibility are carefully considered by Ghiadoni et al.[1] in this issue of the journal.

So what is endothelial function? The endothelium is a physical barrier of cells, attached directly or indirectly to an underlying basement membrane, which separates the vascular intima from the media in larger blood vessels, and the flowing blood from bodily cells in the smallest [2]. It covers a huge area in the body, far larger than the skin, the pulmonary alveolar lining, and the convoluted surface of the gastrointestinal tract [3], perhaps all combined. The endothelium monitors the flowing blood, repelling lipoproteins, cells and platelets, and producing plasminogen activator to break up clots that threaten to adhere [3]. Endothelium also influences components in the vascular wall, of which the best known are the medial smooth muscle cells. The response of smooth muscle cells is tested by the method described by Ghiadoni et al.[1]. Increased flow velocity at the endothelial surface facing flowing blood leads to release of nitric oxide (NO) [1-4]. This diffuses from endothelial cells across the intima, which thickens with age and in atherosclerosis [3,4]), into the media in which it causes relaxation of smooth muscle and dilation of the vessel. The normal purpose is to improve conductivity (reduce resistance) of arteries upstream from an active dilated vascular bed [2]. The effect can be immediate, with activity initiated by exercise, ischemia or reactive hyperaemia, and measurable by arterial dilation [1]. The effect can be persistently enhanced by regular exercise continued over months, but this effect wears off within 1 month of exercise cessation [3,5].
Arterial dilation from smooth muscle relaxation obviously decreases resistance but there is also a beneficial effect on arterial stiffness [3,6]. This is best seen in the ‘muscular’ arteries, but not in predominantly ‘elastic’ arteries such as the thoracic aorta [3,6]. The muscle fibres act as though attached to (i.e. 'in series' with) medial collagen, but in parallel with elastin fibres. Hence muscle relaxation (with dilation) transfers stresses in the wall from the stiffer collagen to less stiff elastin. This explains the higher distensibility of muscular arteries dilated by NO, nitroglycerine and arterial dilators including calcium channel blockers (CCBs), angiotensin converting enzyme inhibitors (ACEIs) and angiotensin blockers (ARBs), in contrast to the increased stiffness of arteries dilated by aging and medial degeneration [3]. Elastic arteries such as the thoracic aorta show more degeneration, stiffening, and dilation with age but show little or no change in stiffness with NO, or with drugs such as nitrates, CCBs, ACEIs, ARBs, [3-7]. Muscular arteries in contrast show less (~2% cf 10% (for the aorta) [3,8,9]) dilation with each beat of the heart and less dilation with age, but greater response to endothelially derived NO, and to nitrates and arterial vasodilators which are so useful in treating isolated systolic hypertension (ISH), caused by aortic degeneration and stiffening [3,9].

Lessons learnt from consideration of endothelial (dys)function thus help us to understand the effects of arterial dilating drugs in ISH [10], the commonest form of hypertension today. But there are subtleties. Although ISH is caused by degeneration and stiffening of the aorta, it is not treatable by destiffening of the aorta, but by ‘destiffening’ of the muscular arteries, and through muscular relaxation and dilation, and transfer of stress to the elastin components of the wall [3,6]. Benefit of these drugs on the arterial wall is not apparent as improved (lower) aortic stiffness (through such measures as carotid-femoral pulse wave velocity (CF PWV), or aortic characteristic impedance, but as reduction in input impedance, apparent phase velocity, wave reflection and central aortic pressure [3,7,9,10]).

There is with age a decrease in conventionally measured endothelial function in the brachial artery [1-4]. This is also seen in other muscular arteries such as the coronaries. In the coronary arteries, flow-mediated dilation may be decreased further or reversed in the presence of atherosclerotic disease [2-4,11,12]. At least some of the aging change, and that with intimal thickening and atherosclerosis may be simply due to the greater distance between the site of production and of action of NO (endothelial and muscular cell respectively), and its diffusion elsewhere. But there are more complex factors in play such as the balance between NO and vasoconstricting agents such as endothelin which can increase arterial tone and cause narrowing or occlusion from spasm. Such mechanisms are part of the ability of a normal muscular artery to narrow or close in the event of trauma to and haemorrhage from the vessel. Ghiadoni et al.[1] confidently refer to impaired endothelial function as a cause of atherosclerosis, and this view is supported by quoted references from major journals. But from the above it could be argued that atherosclerosis may be the cause, rather than the result of impaired capacity of NO to cause arterial dilation on the basis of NO loss between endothelial and muscle cell. A Nobel laureate, to whom this proposal was put, said that it was probably correct but he had not heard it before. Perhaps medical scientists seek an answer to questions, which relate to complex mechanisms and overlook the simple, as in the old tale of ‘the king has no clothes’.
Ghiadoni et al.\textsuperscript{[1]} examined the reproducibility of a standard method for measuring brachial arterial dilation to ischemic response in 135 volunteers aged 20-60 years in seven Italian cities. The protocol was identical in all centers, with subjects healthy and lean (BMI 21), and having no cardiovascular disease or major risk factors, on no drug therapy (including aspirin or oral contraceptive in women) and in the follicular menstrual phase in women. Recordings were taken fasting, with no smoking beforehand and over a 2-3-h period in the morning. Well validated, dedicated echocardiographic equipment was used with specially trained technicians. Stereotaxic equipment was used to adjust and hold the ultrasonic sensor over the brachial artery. Participants were allowed to rest for prolonged periods prior to measurements being taken. Data were analysed centrally. It is not possible to conceive better circumstances for a trial of brachial endothelial function. The study shows what can be achieved under ideal conditions.

Flow-mediated dilation (FMD) was not significantly different on three different occasions (twice on 1 day, and once 30 days later). FMD intra-session averaged 9.9 +/- 8.4% across centres, and inter-session coefficient of variation was 12.9 +/- 11.6%. Authors concluded that rigorous and standardized procedure may provide reproducible FMD assessment to study endothelial function in multicenter clinical trials.

The authors are to be complemented on this study, which is the best study of endothelially derived FMD to be published in the 20 years since the technique was introduced. The results are not as good as many previously had claimed, and explain some of the conflicting results in the literature.

The main question that arises from this work can be phrased in Latin as in the title of this commentary, or with a colloquial 'so what'? Is it possible to achieve similar results in multicenter practice? Is it warranted to undertake such an examination over 2-3 h, with requirement of a skilled experienced technician, micromanipulator, expensive ultrasound equipment, with preliminary fasting, cessation of medication etc.? A vascular MRI examination may be more revealing \textsuperscript{[13]} and cost less.

Further questions relate to other techniques such as analysis of the arterial pulse waveform to give equivalent (or better) information in shorter time at less cost. Tests of aortic stiffening as CF PWV have been shown to predict cardiovascular events independent of brachial blood pressure (BP) \textsuperscript{[14,15]}. Information on the pulse waveform itself on wave reflection and aortic systolic or pulse pressure (PP) likewise predict events independent of brachial BP and probably independent of aortic stiffening \textsuperscript{[15-18]}. But similar issues have arisen on many of the techniques and have caused the type of confusion referred to in Ghiadoni's study of flow-mediated dilation. Many of the pulse wave studies had not been calibrated against a predicate such as required by the US Food and Drug Administration (FDA), or had fundamental errors of mathematics in computer programming, or had
used arterial tonometry in situations wherein it was inappropriate [3,19,20,21]. The Journal of Hypertension has taken a leading role in airing controversy, and in seeking consensus [19-23], and our own viewpoint has been fairly represented [19,22,23]. Further clarification is being sought in relation to conflicting views on wave reflection and outcome [18,24] through reanalysis of the Framingham study with attention directed at the radial rather than the brachial tonometric pulse. All this illustrates the continuing relevance of Carl Ludwig's dictum 'Der Methode ist Alles'.

Ghiadoni and colleagues [1,2,4] question techniques based on analysis of the arterial pressure or flow waveform, noting that these were not accepted by the AHA/ACC taskforce as providing useful information whereas tests of endothelial function do. The AHA/ACC statement [25] has been challenged on the basis of 'experts' not necessarily having the expertise required, having gained their appointment as representatives of professional groups. With respect to arterial stiffness and wave reflection, the AHA/ACC Taskforce collected 'evidence' in the literature from use of key words that did not include the fundamentals such as 'arterial stiffening', 'pulse wave', 'pulse wave velocity (PWV)', 'pulse wave analysis', 'tonometry' or 'wave reflection' [26]. It is interesting to note that the forthcoming US JNC8 statement has been prepared not by representatives of professional groups as in the past but by individuals selected through the US Department of Health on the basis of discipline (Presentation of S. Oparil, International Society of Hypertension (ISH) meeting, Beijing, Nov 2011).

Although these issues are in the process of clarification, they pale into insignificance when one considers the contribution of endothelial dysfunction to cerebrovascular disease. The ultimate in endothelial dysfunction is when endothelial cells are breached, or torn off their basement membrane and shed from the vascular wall.

Lesions which present 'the ultimate' (loss of endothelium cells) are widely described in the older clinical literature on cerebral microvascular disease, with bleeding into and through the endothelium and media, and with micro-haemorrhage and formation of Charcot Bouchard aneurysms in older persons and particularly in those with long-standing hypertension. Ross Russell [27], Fischer [28] and colleagues, 50 or more years back stressed the importance of these cerebral lesions and their role in the development of micro-thrombosis, infarcts, and cerebral haemorrhage. Byrom [29,30], the winner of the ISH Volhard prize in 1976 described these and similar lesions also as arising in the malignant phase of hypertension and caused solely by acute elevation of arterial pressure. He showed narrowing and dilation as occurring in the same small artery subject to high pressure, and their resolution with normalisation of pressure. Areas of narrowing and dilation are attributed to endothelial damage with loss of local vasodilator and vasoconstrictor control. Fry [31,32] showed how endothelial cells could be shed from coronary arteries in particular sites, as a consequence of jets or eddies impacting on the wall. Fry [31] calculated that the shear stress causing such damage to or shedding of endothelial cells was not far above normal shear stress. This has been confirmed more recently and suggested as the cause of microvascular cerebral disease when aortic stiffening with age funnels flow pulsations into cerebral arteries [3,33]. These past findings attract little or no
attention in present vascular medicine or hypertension publications. But neuroradiologists using MRI are finding more evidence of cerebral lesions in older persons with or prior to clinical evidence of cerebral deterioration [34]. Evidence of a link between aortic stiffening and high-flow pulsation is strengthening, with damage to cerebral microcirculation ('pulse wave encephalopathy') increasingly attributed to this [35,36]. The relevance to intellectual deterioration and dementia is becoming a focus of interest [37], whereas neurosurgeons and their intensive care colleagues struggle with late complications, of head injury or stroke (especially subarachnoid haemorrhage) and attributable to spasm [36] in other parts of the brain. These issues question how to best manage patients with raised intracranial pressure, the complications of impaired volume flow, and increased pulsatile flow as a consequence of vascular compression [38,39].

These issues of cerebral microvascular damage are relevant to renal damage as well [30,33], because both organs have high pulsatile as well as steady flow, and microvessels are not protected by high-resistance arteries and arterioles upstream, when aortic stiffness increases with age. The primary insult appears to be from within the blood vessel, and on the endothelium. The perverted balance of vasodilatory and vasoconstrictor mechanisms [29] appears responsible for the excessive dilation which predisposes to rupture [3,33] and the constricted areas of apparent spasm which cause further ischemia. The endothelium is intimately connected with cerebral and renal dysfunction with age. New approaches to the study of endothelial function in the brain and kidney are likely to be very valuable in reducing the burden of stroke and of dementia in our communities.

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Conflicts of interest

M.O'R. is a founding director of AtCor Medical, manufacturer of systems for analysing the arterial pulse and of Aortic Wrap Pty Limited, a developer of devices to improve aortic distensibility.

REFERENCES


17. Vlachopoulos C, Aznaouridis K, O'Rourke MF, Safar ME, Stefanadis C. Towards the final verdict: re central pulse pressure vs peripheral pulse pressure: case still open? Eur Heart J 2010; April 11 letter to the editor (online only).


38. Eide PK, Bentsen G, Sorteberg AG, Marthinsen PB, Stubhaug A, Sorteberg W. A randomized and blinded single-center trial comparing the effect of intracranial pressure and intracranial pressure wave amplitude-