

Evaluation of arterial endothelial function using transit times of artificially induced pulses

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Abstract

Impairment of arterial endothelial function is an early event in atherosclerosis and correlates with the major risk factors for cardiovascular disease. The most widely employed non-invasive measure of endothelial function involves brachial artery (BA) diameter measurement using ultrasound imaging before and after several minutes of blood flow occlusion. The change in arterial diameter is a measure of flow-mediated vasorelaxation (FMVR). The high between-laboratory variability of results and cost of instrumentation render this technique unsuitable for routine clinical use. We induce artificial pulses at the superficial radial artery using a linear actuator. An ultrasonic Doppler stethoscope detects these pulses 10–30 cm proximal to the point of pulse induction. The delay between pulse application and detection provides the pulse transit time (PTT). By measuring PTT before and after 5 min of BA occlusion and ensuing reactive hyperemia, FMVR may be measured based on the changes in PTT caused by changes in vessel caliber, smooth muscle tone and wall thickness. We (1) validate the sensitivity of this technique to arterial wall tone using sublingual nitroglycerin and (2) compare measurements of endothelial function to ultrasound BA diameter measurements in 12 human subjects. The PTT-based method is verified to measure arterial wall tone and is shown to provide 37% greater sensitivity ($p < 0.05$) to FMVR than BA diameter measurements. By measuring the change in pulse transit time before and after endothelial stimulus, a sensitive, reproducible and convenient measure of endothelial function may be obtained at a low cost.

Keywords: endothelium, atherosclerosis, ultrasonics, nitric oxide, muscle, smooth, pulse wave velocity, pulse transit time, reactive hyperemia

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Endothelial dysfunction (ED) is an important initial event in atherogenesis (Zeiber *et al* 1991, Celemajer *et al* 1992) and is strongly correlated with all the major risk factors for cardiovascular disease (CVD) (Vita *et al* 1990). In addition, it is one of the earliest predictors of CVD (Vita *et al* 1990, Celermajer *et al* 1994) and coronary events (Neunteufl *et al* 2000). Conversely, factors such as exercise appear to improve endothelial function (EF) (Higashi *et al* 1999). This research is motivated by the need to incorporate a convenient and reliable measurement method for the assessment of EF into the routine medical examination.

The most common technique for the assessment of EF is the measurement of the diameter of the brachial artery (BA) using ultrasound before and after an arterial occlusion of several minutes duration. The reactive hyperemic flow (RHF) that ensues once the occlusion is removed increases the shear stress on the arterial wall and thereby stimulates endothelial cells to release chemical factors that relax the surrounding vascular smooth muscle. The change in arterial caliber effected by this mechanism is termed as flow-mediated vasodilation (FMD).

In human arteries, FMD appears to be mediated primarily by the action of endothelium-derived nitric oxide (NO) on vascular smooth muscle (SM). Generation of NO is dependent on the activation of the enzyme endothelial nitric oxide synthase (eNOS). Inhibition of this enzyme abolishes FMD in these arteries (Lieberman *et al* 1996). Acute changes in the concentration of eNOS are in turn dependent on the influx of Ca^{2+} into the cell. Shear stress on the endothelial cell wall appears to open Ca^{2+} -activated K^{+} channels thereby hyperpolarizing the cell and facilitating Ca^{2+} influx (Corretti *et al* 2002). A Ca^{2+} -independent tyrosine kinase-dependent mechanism is also involved (Ayajiki *et al* 1996). NO stimulates the release of soluble guanylyl cyclase in the SM which in turn increases generation of cyclic guanosine monophosphate (GMP). Hyperpolarization of the SM and consequent inhibition of contraction occurs via cGMP-dependent protein kinase phosphorylation of K^{+} channels (Mombouli and Vanhoutte 1999). After the cessation of vasodilatory stimulus (such as shear stress), the remaining cGMP is hydrolyzed by phosphodiesterases within minutes, restoring basal SM tone. Maintenance of high levels of shear stress for many minutes or hours leads to transcription of the eNOS gene and hence increased NO production and prolonged vasorelaxation (Corretti *et al* 2002).

EF in peripheral arteries is a valid target for clinical measurements as it is representative of global EF. FMD in the BA correlates strongly ($r = 0.78$ (Takase *et al* 1998)) with dysfunction of the coronary endothelium. A BA FMD of less than 3% appears to have a positive predictive value of 95% for coronary ED (Anderson *et al* 1995). FMDs of less than 4.5% have a sensitivity of 70% and a specificity of 80% for angiographically-detectable coronary artery disease (CAD) (Enderle *et al* 1998), while FMDs greater than 10% have a 95% negative predictive value for CAD (Neunteufl *et al* 2000). In cases of advanced CAD (greater than 50% stenosis), FMD values less than 6% are found to be predictive with a sensitivity of 93% and a specificity of 88% (Teragawa *et al* 2001).

Since ED is thought to be a necessary condition for the development of atherosclerosis, measurement of FMD by ultrasonic imaging has the potential to yield important diagnostic information. However, the coefficients-of-variation (CVs) of the FMD measurements obtained using the ultrasound imaging method vary widely from as little as 1.5% in very carefully conducted studies to approximately 50% in others (Sorensen *et al* 1995, Andrews *et al* 1997, Hardie *et al* 1997, de Roos *et al* 2001). Some investigators have considered FMD values of less than 5% as indicative of ED, while others have observed mean FMDs of more than 5% in atherosclerotic subjects. Differences in methodology are most likely responsible for the large range of reported CVs. Although adherence to recently published standardization

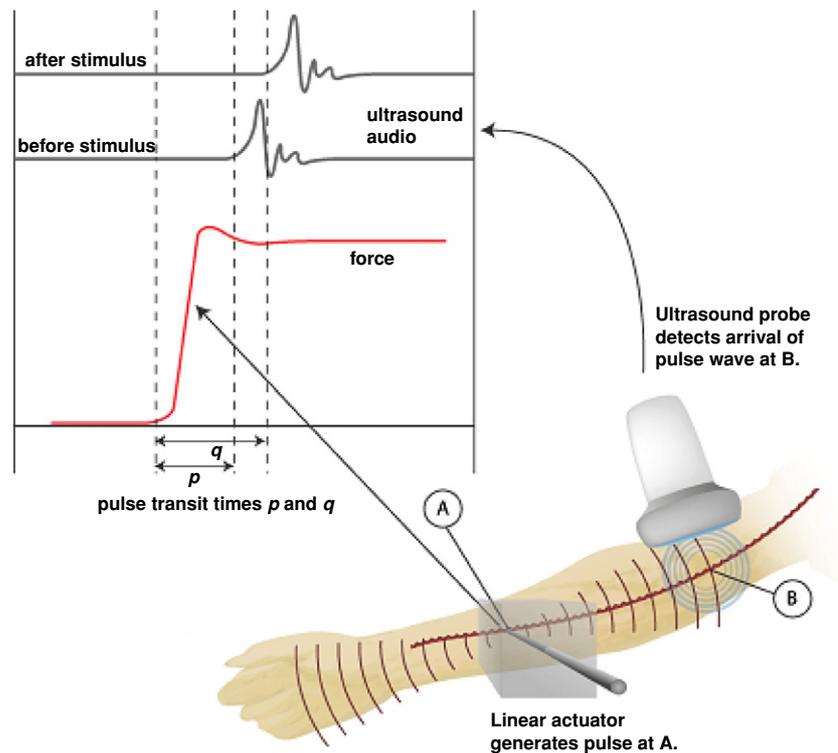


Figure 1. Vasorelaxation is quantified by the ratio (q/p) of measured transit times of artificial pulses before and after a stimulus.

guidelines (Corretti *et al* 2002) will reduce inter- and intralaboratory study variance, the intrinsic complexity of BA diameter measurement remains. A simpler technique that is less sensitive to sources of error such as subject motion would be desirable.

It is important from the outset to clearly distinguish the variability due to the BA diameter measurement method and the inherent variability of EF. Were the latter characterized by high variability, there would be little purpose in proposing a new instrument for its measurement. A comprehensive analysis of the variability of carefully conducted BA diameter measurements appears in (Sorensen *et al* 1995). The overall CV in this study of 40 subjects examined four times each was 1.8%. This evidence lends great support to the contention that it is possible to obtain reproducible measurements of EF via observations of flow-mediated vasorelaxation (FMVR).

We have developed a new instrument for the non-invasive assessment of FMVR, which we refer to as the 'vascular relaxoscope'. The relaxoscope measures vasorelaxation via the effect of this process on the pulse transit time (PTT) of an artificial pulse through an arterial segment. Figure 1 illustrates this schematically.

Many techniques for PTT measurement have been presented in the past (Nichols and O'Rourke 1998, pp 64–7). Most calculate the propagation delay of the rising edge (foot) of the natural pulse between two points along the vessel. Non-linear viscoelastic properties of arterial walls introduce dispersion. Different frequency components of the wave thus travel at different speeds, reducing timing accuracy.

Anliker *et al* (1968) measured PTT by inducing artificial pulse waves within the canine aorta using a vibrating catheter. An intra-arterial manometer detected the traveling waves.

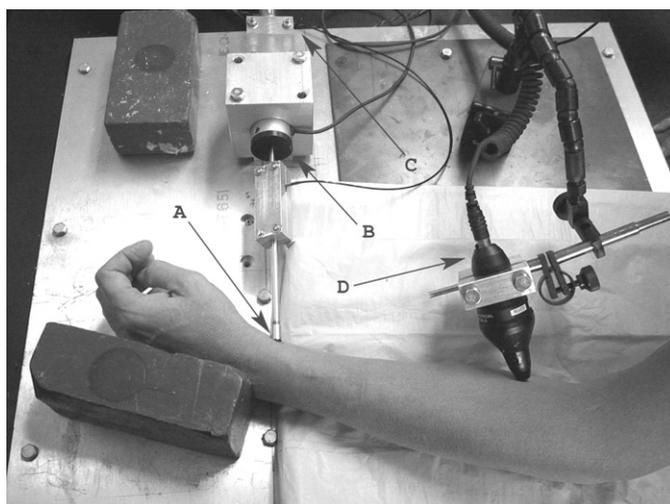


Figure 2. A subject's arm is shown positioned on the relaxoscope platform. Artificial pulses are applied by the actuator at (A), which is driven by linear motor (B). These are detected by an ultrasound probe (D). Actuator displacement is measured by a linear variable differential transformer (C).

Measurements were superior to those obtained using natural pulses, owing to the high definition of the low-amplitude artificial pulses. Landowne performed similar measurements in the human forearm. An armature delivered mechanical pulses to the radial artery, and resultant pressure waves were measured proximal to the site of administration using an intra-arterial sensor (Landowne 1957). The relaxoscope, which we now describe, performs this type of measurement using non-invasive pulse induction and detection.

A preliminary description of the relaxoscope and its application to the measurement of endothelial function in humans appears in (Maltz and Budinger 2003). Measurement accuracy and reproducibility were not addressed. In this paper, we comprehensively describe the instrument and provide a thorough physiological justification for the measurement procedure. In addition, we examine the linearity of the PTT measurement versus propagation distance, calculate the sensitivity of the measured PTT to changes in arterial tone, perform intra- and interobserver studies to determine coefficients-of-variation and describe sources of measurement error. We also propose technical and ergonomic improvements to the device.

2. Methods

2.1. Instrumentation

Figure 1 illustrates the artificial pulse induction and PTT measurement scheme. The linear actuator induces an artificial pulse at the superficial radial artery and detects this pulse at a point 10–30 cm proximal, using an ultrasonic flow meter.

The current prototype of the apparatus appears in figure 2. The instrument employs a linear motor (designed to our specification by Baldor Electric Co., Fort Smith, AR), the actuating stem of which makes contact with the skin to introduce an artificial pulse. An applanation tonometer (SPT301, Millar Instruments, Inc., Houston, TX) at the free end of the stem senses the applied force. This allows for closed-loop control of the force waveform and

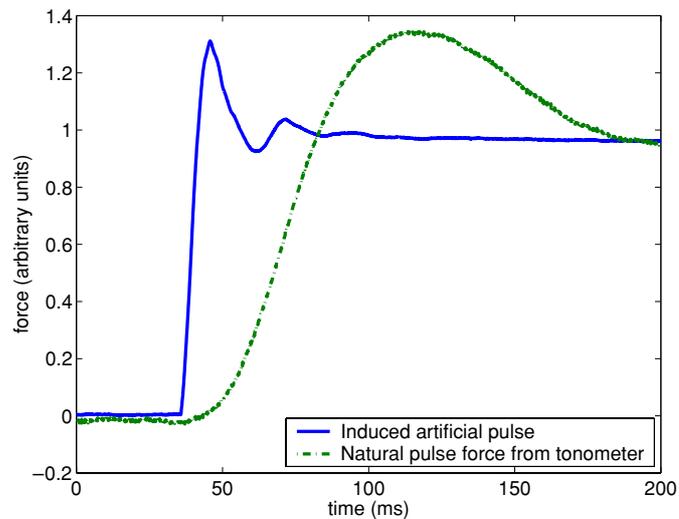


Figure 3. The foot of the artificial pulse may be precisely determined to within 1 ms. The corresponding uncertainty for the natural pulse is ≈ 10 ms. Transit times of 10–20 ms cannot be accurately resolved using the natural pulse. Pulses have been scaled to similar amplitude.

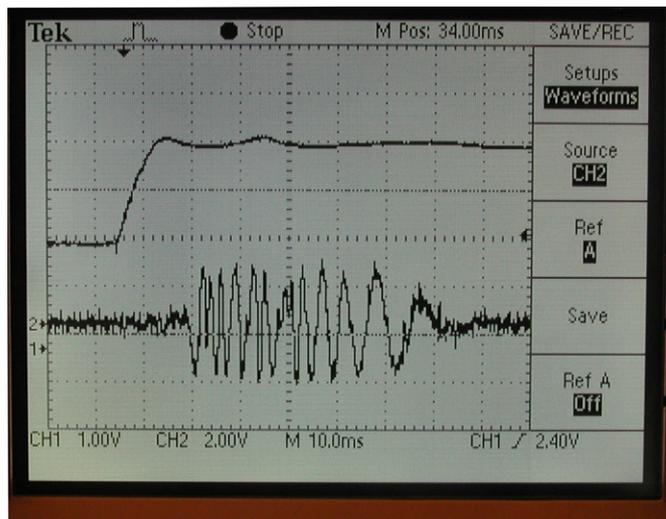


Figure 4. The upper and lower oscilloscope traces show the respective rising edges of the applied force waveform and the received ultrasound signal. In this example, the foot–foot delay is approximately 14 ms.

accommodates different lateral positions of the wrist of the subject. Figure 3 compares the rising edges of typical artificial and natural pulses.

An 8 MHz continuous wave (CW) Doppler ultrasonic stethoscope (Imex Pocket-Dop II, Nicolet Vascular, Madison, WI) or a 7.5 MHz pulsed wave (PW) imaging probe (AU5, Biosound Esaote Inc., Indianapolis, IN) records the flow waves associated with these pulses. Figure 4 illustrates real-time waveforms of the force applied to the wrist and the received Doppler signal during a human subject study.

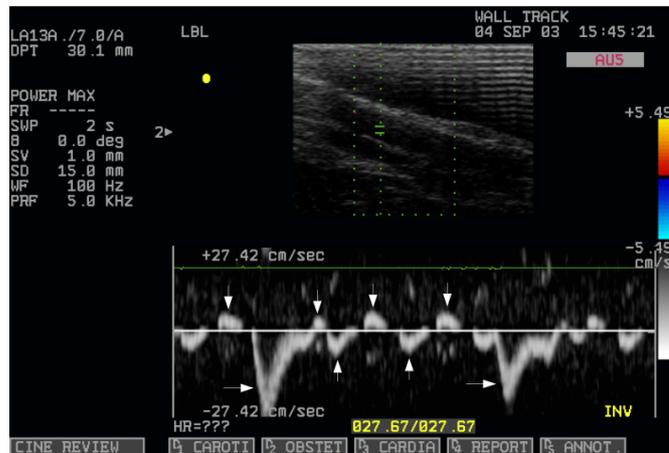


Figure 5. Sonogram of flow in the brachial artery 27 cm proximal to the point of pulse induction. Positive amplitude pulses (down arrows) represent the flow generated on the incident stroke of the actuator. Negative pulses are generated on the retreating stroke (up arrows) and by the heart (right arrows).

Figure 5 shows the sonogram of a typical observed waveform. This recording was taken in the BA. The pulse induction frequency was 3 Hz. This figure serves as strong evidence that the detected pulse travels along the artery and not through other tissues.

The force input and ultrasound audio signals are digitized (NI-PCI 6035E, National Instruments Corp., Austin, TX) at a sampling rate of 10 kHz per channel.

At present, PTT measurements are obtained either manually by marking the foot of the induced force pulse (applied at point A in figure 2) and the foot of the ultrasound flow signal detected at transducer D, or semiautomatically by using a supervised thresholding algorithm.

Measurements taken during periods where the natural pulse is present are discarded as the natural pulse obscures the rising edge of the artificial pulse waveform. A second reason why these measurements are discarded is that the large amount of fluid flow during the systolic pulse alters the PTT by a small amount (Hiland and Anliker 1973).

2.2. Evaluation of arterial EF

The Moens–Korteweg equation provides physical justification for the use of PTT to measure changes in arterial wall tone and hence FMVR. It relates the PTT over a distance d

$$PTT = d \sqrt{\frac{2\rho r}{Eh}} \quad (1)$$

to the arterial wall Young's modulus (E), arterial wall thickness (h), arterial radius (r) and the blood density (ρ). As SM relaxes in response to NO released by the endothelium, r increases while E and h both decrease. PTT consequently always increases upon SM relaxation (Nichols and O'Rourke 1998, p 63). The proportional increase in PTT may thus be greater than that for diameter alone.

2.3. Human studies

We performed the following experiments to validate the relaxoscope.

- (i) We measured PTT as a function of estimated distance between the actuator and the probe to verify the incremental linearity of our PTT measurements.
- (ii) We evaluated PTT measurement reproducibility via inter- and intra-observer studies.
- (iii) We confirmed that the PTT measurements are sensitive to arterial wall relaxation induced by sublingual nitroglycerin (NG).
- (iv) We confirmed that the reactive hyperemia (RH) induces the predicted increase in PTT and that this increase is correlated with that of BA diameter following an identical stimulus.

Protocols were approved by the university institutional review board, and experiments were conducted in compliance with institutional guidelines. Subjects gave informed consent to participate in all experiments.

All experiments were performed in a temperature-controlled room. Each subject was given at least 10 min to acclimate to the posture assumed during measurement.

Blood pressure (BP) was measured at the BA before and after each procedure. Readings were taken on the arm contralateral to that upon which the most recent procedure was performed.

2.3.1. PTT as a function of propagation distance. PTT measurements were taken in two subjects with the ultrasound probe positioned at three locations along the radial and brachial arteries. The position of pulse induction remained constant. The distance between the actuator tip and the range gate of the PW probe was estimated using a stiff wire and B-mode imaging to trace the course of the artery.

2.3.2. Inter- and intra-observer studies. Absolute PTT measurements were obtained from two subjects by three observers. Each observer examined each subject three times in succession.

In the absence of the other observers, the first observer positioned the actuator and probe and obtained PTT recordings. The depth of the artery and the lateral location of the range gate on the B-mode display were recorded. Three photographs were then taken of the subject's arm, and the straight-line distance between the actuator and probe was measured using a rule. Observers 2 and 3 were then given the photographs and measurements and told to place the actuator and probe as depicted while ensuring that the separation distance was equal to that specified.

2.3.3. Sensitivity of PTT measurements to arterial wall properties. We sought to verify that the PTT change measured by the relaxoscope is indeed a reproducible measure of arterial wall tension. To determine the intrinsic variability of a differential measure, a stimulus must be employed. This stimulus should contribute as little as possible to the measurement variance. To this end, we employed sublingual NG as a vasodilatory stimulus since it generates NO that acts directly on vascular SM to produce vasorelaxation. As this is an endothelium-independent process, the results are less dependent on factors such as time of day, fasting status and medication status (Corretti *et al* 2002). This facilitates the acquisition of intrinsic reproducibility data.

Baseline PTT measurements were acquired for 1 min from the left forearm. Sublingual NG (0 mg or 0.6 mg) was then administered while the arm remained stationary. Measurement of PTT response ensued for 7 min.

The maximum response to NG was calculated as

$$\text{PTT}_{\text{fracMax}} = \max_{t > t_s} \text{PTT}_{\text{frac}} = \frac{\max_{t > t_s} \{\text{PTT}(t)\}}{\text{PTT}_0}, \quad (2)$$

where $t_s = 60$ s is the time of stimulus. The mean baseline PTT is denoted as PTT_0 . The metric in (2) is chosen in order to facilitate comparison with values cited in the literature.

Since three experiments were performed on each subject for each of the two treatments (0 mg NG and 0.6 mg NG), multivariate analysis of variance (MANOVA) is employed to analyze the data. The MANOVA treated the subject response on each day as a dependent variable. A custom implementation of MANOVA was coded in Matlab 5.3 (The Mathworks, Inc., Natick, MA) (Christensen 2001, pp 33–40). The Lawley–Hotelling trace test was used to evaluate the null hypothesis of equal treatment means. A significance level of $\alpha = 0.05$ was selected.

2.3.4. Sensitivity of PTT measurements to flow-induced, endothelium-mediated vasorelaxation. We determined whether the change in PTT before and after stimulation of the endothelium correlates with the change in artery diameter as predicted by (1). Two procedures were performed in random order.

Procedure A. FMD was stimulated using a 5 min suprasystolic BA cuff occlusion. Right arm BA diameter was measured using the ultrasonic imager described in section 2.1 equipped with an M-mode wall echo-tracking device (Wall Track System II, Pie Medical, Maastricht, Netherlands) before and for 5 min after cuff release. The subjects remained supine throughout the study.

The maximum change in BA diameter, which usually occurs between 1 and 2 min after cuff release (Berry *et al* 2000), was calculated as

$$r_{\text{fracMax}} = \max_{t > t_s} r_{\text{frac}} = \frac{\max_{t > t_s} \{r(t)\}}{r_0}.$$

Procedure B. With the subject seated at the relaxoscope, baseline PTT measurements were acquired for 1 min. The cuff was then inflated for 5 min. Upon cuff release, recording of signals ensued for 5 min.

The two procedures were carried out sequentially on alternate arms in random order on an individual subject, with a minimum and maximum respective intervals of 30 and 60 min between the procedures.

3. Results

3.1. PTT as a function of propagation distance

Correlations of 1.00 and 0.97 were observed between PTT and propagation distance in the two subjects examined.

3.2. Inter- and intra-observer studies

The intra-observer and inter-observer CVs were 3.2% and 2.7%, respectively.

3.3. Nitroglycerin studies

Each of the four subjects was examined on six days. On three of these days, 0.6 mg of NG was administered. On the three other days, subjects received no sublingual NG.

Table 1 summarizes the observed effects of treatment with 0 mg and 0.6 mg NG. A large, highly significant difference in PTT_{fracMax} and a smaller, though still significant difference in

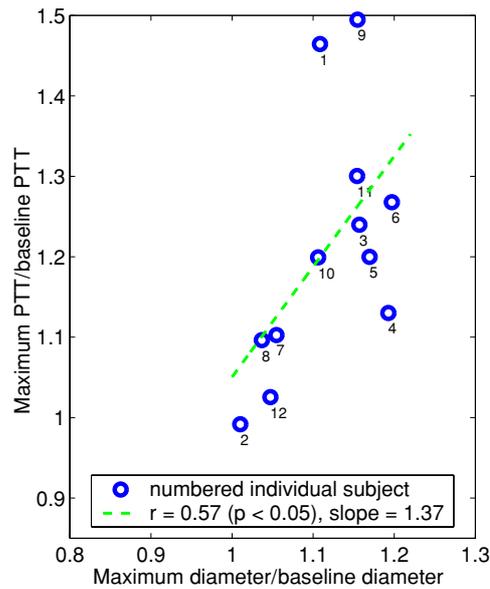


Figure 6. Maximum pulse transit time versus maximum diameter as a fraction of their respective baseline values following reactive hyperemia.

Table 1. Effects of NG treatment on PTT and BP.

Mean quantity \pm SEM	0 mg NG ($N = 12$)	0.6 mg NG ($N = 12$)	p value
Maximum PTT/baseline (PTT_{fracMax})	1.05 ± 0.02	1.21 ± 0.02	*0.0036
Final PTT/baseline ($PTT_{\text{fracFinal}}$)	1.01 ± 0.03	1.06 ± 0.02	*0.0433
Final systolic BP/baseline	0.99 ± 0.02	0.98 ± 0.02	0.3325
Final diastolic BP/baseline	0.94 ± 0.03	0.89 ± 0.04	0.0634
Coefficient-of-variation of PTT_{fracMax} (%)	4.0 ± 1.7	4.2 ± 0.9	

* Denotes statistical significance at $\alpha = 0.05$ level. Bp: blood pressure, SEM: standard error of the mean, NG: nitroglycerin, PTT: pulse transit time.

$PTT_{\text{fracFinal}}$ are observed between the two treatment groups. Final BP values did not differ significantly from initial measures of these quantities.

CVs were close to 4% in both of the treatment groups.

3.4. Comparison with arterial diameter measurement for assessing EF

Twelve human subject studies were performed under the protocol described in section 2.3.4. In figure 6, the ratio PTT_{fracMax} is plotted against r_{fracMax} . A regression line was fitted using the unweighted least-squares method.

4. Discussion

We have demonstrated a new non-invasive method of assessing changes in arterial tone via measurement of the propagation times of artificial pulses. Our experimental results confirm the theoretical prediction that the measurement is considerably more sensitive to vasorelaxation than BA diameter measurement using ultrasound.

Our PTT measurements could not be compared to those obtained using an existing method, as methods based on the natural pulse do not have sufficient timing resolution over short arterial segments (a typical natural pulse has a rise time of 100 ms versus 10 ms for the relaxoscope). We verified instead the strong incremental linearity of the measured PTT as a function of the pulse propagation distance. Using PW Doppler, we confirmed that the artificial pulse does indeed travel along, and is detected within, the artery. These two observations prove that the relaxoscope measures incremental PTT. Our inter- and intra-observer studies show that these measurements are highly reproducible, with both inter- and intra-observer CVs below 3.5%. In view of this evidence we do not describe initial phantom validation studies of the relaxoscope.

Blood flow in the radial artery affects the measured PTT. Increases in fluid flow rate increase the PTT as measured by the relaxoscope so it is possible that flow increases associated with reactive hyperemia increase PTT independent of the tone of the arterial wall. We estimate, however, that any errors induced in this way are negligible. Let us assume a worst-case scenario in which flow is elevated to five times the mean during PTT measurement (a factor of 3 is realistic based on the results of Berry *et al* (2000)). Since, as discussed in section 2.1, measurements taken during the systolic pulse or reflections thereof are discarded, we consider the mean flow rate in this discussion. The typical mean flow rate in the radial artery is 0.1147 ml s^{-1} (Moore *et al* 2002). A typical artery diameter is 2.7 mm (Hosono *et al* 2000). The mean blood velocity is thus 20 mm s^{-1} . The PWV in the radial artery is of the order of 10 m s^{-1} in humans (Nichols and O'Rourke 1998, p 92). Thus, even a five-fold increase in flow rate produces an error of less than 1% in the measured PTT in this artery.

Our NG studies addressed the issue of the reproducibility of differential PTT (dPTT) measurements. Since subject response to NG is not always identical, these studies provide an upper bound on intrinsic variability of dPTT. In studies involving four subjects examined three times each, day-to-day CVs were close to 4% regardless of NG dose (0 mg or 0.6 mg). We are encouraged by these results, since a much larger NG BA diameter study (40 subjects) reported a CV of 2.8% (Sorensen *et al* 1995).

We believe that the above data strongly support the contention that the relaxoscope is a powerful tool for measuring dPTT. In addition, we found that maximum dPTT correlates significantly with the maximum differential change in artery diameter r_{fracMax} ($r = 0.57$, $p < 0.05$, $N = 12$) following RH. This is exactly what is predicted by the Moens–Korteweg equation. Regression analysis showed that the relaxoscope is 37% more sensitive to FMVR than diameter measurements. A limitation of this set of experiments is that only one study per subject was conducted. However, our NG studies already validate the reproducibility and sensitivity of our dPTT measurements to vasorelaxation. We expect any additional variability in the FMVR measurements over the post-NG measurements to be small, since carefully conducted BA diameter studies yield within-subject CVs of 1.8% for FMVR versus 2.8% for NG (Sorensen *et al* 1995).

Among the 12 subjects evaluated, the four whose FMD responses as measured by BA diameter change were indicative of ED (<10%) also exhibited the smallest increases in PTT.

Using upper arm occlusion to induce RH in FMD studies has been criticized (Ramsey *et al* 1996) on the basis that the diameter change is measured in an arterial segment subjected to several minutes of ischemia. Subsequent changes in arterial tone might thus be due in part to ischemic response in addition to shear stress-induced response. The consensus is that this criticism is largely unsupported by the data (Blair *et al* 1959, Carlsson *et al* 1987, Engelke *et al* 1996, Agewall *et al* 2001). Firstly, total RHF typically exceeds flow debt by approximately 500% (Berry *et al* 2000). This flow most likely restores the original hemodynamic properties of the blood in the forearm, especially since venous flow is not

occluded during RH. As a result, any chemicals released during ischemia have most likely washed out by the time peak vasorelaxation is measured between 45 s and 2 min after RH begins. Secondly, most arterial diameter studies observe a constriction of the artery relative to baseline immediately following cuff release (Berry *et al* 2000). This is inconsistent with the conjecture that vasodilatory substances present under ischemic conditions materially affect arterial tone after systemic blood pressure and flow is restored to the vessel. Thirdly, the dilation of the BA due to RH following upper arm occlusion is abolished after prior administration of the eNOS inhibitor NG-monomethyl-L-arginine (L-NMMA) (Lieberman *et al* 1996). If upper arm occlusion-induced ischemia were indeed responsible for increased dilation of the BA by any mechanism that did not involve eNOS, this diameter increase would be preserved after L-NMMA administration. Since studies that have compared upper and lower arm occlusion have found that upper arm occlusion induces greater RHF and greater diameter increase (Berry *et al* 2000, Agewall *et al* 2001), it is likely that FMD following upper arm occlusion is a more sensitive measure of NO-related endothelial function.

Another criticism that applies to both the relaxoscope and diameter measurement methods is that without a measure of the shear stress on the endothelium, it is possible that decreased FMVR may be due to impaired reactive hyperemic response rather than endothelial dysfunction. In many ultrasound diameter studies, peak reactive hyperemic flow is measured using Doppler methods. However, we are not aware of any studies that employ a model to accommodate this confounding effect. Correlations between FMD and disease states are made without any compensation for the magnitude of RHF. A possible reason for this is that peak RH has higher variability than FMD. Also, it is difficult to identify what constitutes a normal reactive hyperemic response because it is dependent on sex and forearm composition (Wascher *et al* 1998). In addition, the relationship between peak hyperemic flow and wall shear stress is not constant. Flow pulsatility and changes in flow velocity profile over the course of RH imply a highly non-stationary relationship between these two quantities. At present it is not practical to measure the parameters that would be required for a useful model. This is not to suggest that such a model could not be derived as the subject of a future investigation. Artery diameter studies that have exhibited the lowest within-subject CVs (the best reported being as low as 1.8%) have employed long occlusion intervals of 5 min and have quantified FMD using the maximum dilation during the post-occlusion interval (Sorensen *et al* 1995). Five minute occlusions tend to produce RHF well in excess of that needed to obtain full endothelial stimulation. This explains why the between-subject CVs of FMD are much lower than the those of RHF. Typical between-subject CVs for peak RHF are approximately 10%, while those for maximum FMD are 3.5% (Berry *et al* 2000).

There can be no doubt that failure of downstream resistance vessel beds to dilate will lead to impaired RH and hence decreased FMVR. If this failure is not related to the function of the endothelium, then FMD and FMVR measurements will lead to a false positive diagnosis of endothelial dysfunction. Several mechanisms have been identified that contribute to RH. The primary dilation appears to be mediated via myogenic mechanisms that are entirely endothelium independent (Carlsson *et al* 1987). Impaired myogenic response would most likely be encountered in subjects with advanced sclerosis of peripheral vascular beds. These subjects, and perhaps subjects with sickle cell disease, would produce false positives for ED. Several endothelium-mediated mechanisms appear to be involved in RH (Engelke *et al* 1996). These involve NO (Tagawa *et al* 1994, Joannides *et al* 1995, Meredith *et al* 1996, Dakak *et al* 1998), prostaglandins (Engelke *et al* 1996), adenosine (Costa *et al* 1999), endothelium-derived hyperpolarizing factors (EDHFs) (Corretti *et al* 2002), potassium ions and pH. Impaired RH is thus strong evidence of either peripheral vascular disease or endothelial dysfunction. Some studies show that RH is moderately impaired in subjects with cardiovascular disease risk

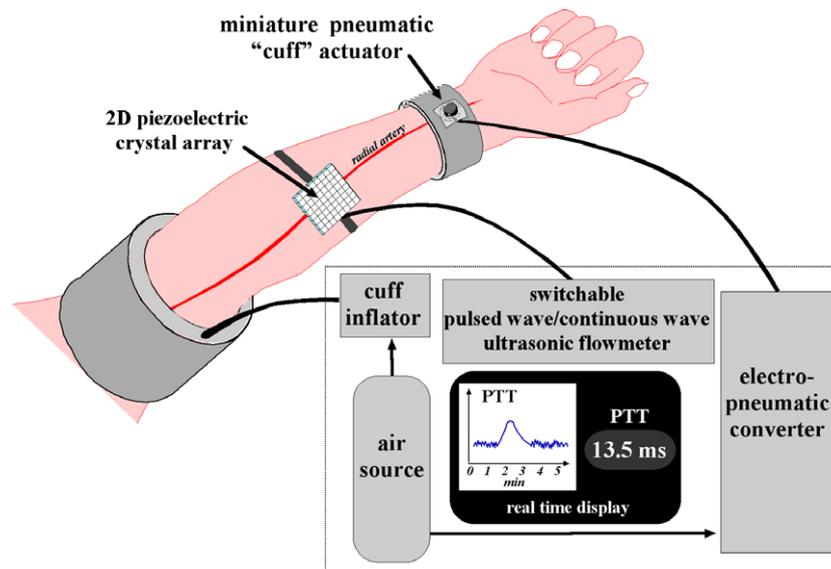


Figure 7. Conceptual design of the second-generation relaxoscope. Artificial pulses are induced at the radial artery by a miniature pneumatic 'cuff' coupled to an electro-pneumatic converter. A 2D array of piezoelectric crystals transmits and receives ultrasound signals. A microprocessor coordinates cuff inflation, deflation, automatic artery location and real-time display of pulse transit time values.

factors (Vuilleumier *et al* 2002). In a very carefully controlled study, Dakak *et al* found that peak RH in the femoral artery was not impaired in the presence of atherosclerosis or CVD risk factors, but that the part of the RH response that was inhibited by the NO synthase inhibitor L-NMMA (an endothelium-dependent component) was reduced in these subjects as compared to healthy controls (Dakak *et al* 1998). Consequently, it is apparent that the magnitude of RH is itself a measure of endothelial function, albeit one of low sensitivity. Normalizing study results based on peak RH response would thus most likely decrease the sensitivity of FMD and FMVR to endothelial dysfunction. In clinical studies, it is the raw FMD values that have not been corrected for variations in peak RHF that have been shown to have high sensitivity and specificity for CVD and its risk factors (Neunteufl *et al* 2000, Takase *et al* 1998, Anderson *et al* 1995, Enderle *et al* 1998, Teragawa *et al* 2001). In this paper we merely propose an improved method for obtaining these useful measurements, regardless of whether reduced RH is involved in the reduced FMD or FMVR observed. Our experimental data would have been strengthened if peak RHF had been quantified, since it would have at least been possible to control for very low peak RHF. Indeed, measurement of peak RHF may easily be incorporated into the relaxoscope protocol using the existing instrumentation. However, since all subjects were normotensive and had no history of vascular disease, we do not believe differences in RH contributed significantly to our maximum fractional PTT increase (PTT_{fracMax}) values. Furthermore, since the correlation between PTT_{fracMax} and the maximum fractional arterial radius increase (r_{fracMax}) was determined by applying the same ischemic stimulus to the same subject on the same day, this criticism is unlikely to apply to our correlation and sensitivity results.

Our primary goal is to provide researchers and clinicians with a convenient, low-cost tool to enable the evaluation of EF. Our largest inter-observer CV of 3.25% was achieved by three

observers, two of whom had received only 30 min of training and had no previous experience with ultrasonic imaging or flow measurements. This suggests the ease with which reliable relaxoscope measurements are obtained. However ergonomic and technological refinements are needed for this method to gain wide clinical use. Figure 7 illustrates the conceptual design of an instrument suitable for clinical studies. For more convenient pulse induction, the linear actuator is replaced with a wrist bracelet containing a miniature pneumatic cuff that is coupled to a high-bandwidth electropneumatic converter (EPC). Such a system with a bandwidth of 35 Hz has already been developed for BP measurement via the volume-compensation method (Tanaka *et al* 2003). The induced pulses are detected by an array of piezoelectric crystals mounted on a forearm strap. The use of a 2D array enables an automated algorithm to locate the artery, obviating the need for precise sensor placement. Peak hyperemic flow will be recorded so that investigators can consider this variable in their analyses. An embedded microprocessor coordinates the measurement protocol for the evaluation of EF by controlling data acquisition, cuff inflation and release, the EPC and the ultrasonic flow meter parameters.

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References

- Agewall S, Doughty R N, Bagg W, Whalley G A, Braatvedt G and Sharpe N 2001 Comparison of ultrasound assessment of flow-mediated dilatation in the radial and brachial artery with upper and forearm cuff positions *Clin. Physiol.* **21** 9–14
- Anderson T J, Uehata A, Gerhard M D, Meredith I T, Knab S, Delagrangre D, Lieberman E H, Ganz P, Creager M A and Yeung A C 1995 Close relation of endothelial function in the human coronary and peripheral circulations *J. Am. Coll. Cardiol.* **26** 1235–41
- Andrews T, Whitney E, Green G, Kalenian R and Personius B 1997 Effect of gemfibrozil +/- niacin +/- cholestyramine on endothelial function in patients with serum low-density lipoprotein cholesterol levels <160 mg/dl and high-density lipoprotein cholesterol levels <40 mg/dl *Am. J. Cardiol.* **80** 831–5
- Anliker M, Histan M and Ogden E 1968 Dispersion and attenuation of small artificial pressure waves in the canine aorta *Circ. Res.* **23** 539–51
- Ayajiki K, Kindermann M, Hecker M, Fleming I and Busse R 1996 Intracellular pH and tyrosine phosphorylation but not calcium determine shear stress-induced nitric oxide production in native endothelial cells *Circ. Res.* **78** 750–8
- Berry K, Skyrme-Jones R and Meredith I 2000 Occlusion cuff position is an important determinant of the time course and magnitude of human brachial artery flow-mediated dilation *Clin. Sci. (Colch)* **99** 261–7
- Blair D, Glover W, Greenfield A and Roddie I 1959 The increase in tone in forearm resistance blood vessels exposed to increased transmural pressure *J. Physiol.* **149** 614–25
- Carlsson I, Sollevi A and Wennmalm A 1987 The role of myogenic relaxation, adenosine and prostaglandins in human forearm reactive hyperaemia *J. Physiol.* **389** 147–61
- Celermajer D, Sorensen K, Bull C, Robinson J and Deanfield J 1994 Endothelium-dependent dilation in the systemic arteries of asymptomatic subjects relates to coronary risk factors and their interaction *J. Am. Coll. Cardiol.* **24** 1468–74
- Celermajer D, Sorensen K, Gooch V, Spiegelhalter D, Miller O, Sullivan I, Lloyd J and Deanfield J 1992 Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis *Lancet* **340** 1111–5
- Christensen R 2001 *Advanced Linear Modeling* 2nd edn (Berlin: Springer)

- Corretti M C *et al* 2002 Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the international brachial artery reactivity task force *J. Am. Coll. Cardiol.* **39** 257–65
- Costa F, Sulur P, Angel M, Cavalcante J, Haile V, Christman B and Biaggioni I 1999 Intravascular source of adenosine during forearm ischemia in humans: implications for reactive hyperemia *Hypertension* **33** 1453–7
- Dakak N, Husain S, Mulcahy D, Andrews N P, Panza J A, Waclawiw M, Schenke W and Quyyumi A A 1998 Contribution of nitric oxide to reactive hyperemia: impact of endothelial dysfunction *Hypertension* **32** 9–15
- de Roos N, Bots M and Katan M 2001 Replacement of dietary saturated fatty acids by *trans* fatty acids lowers serum HDL cholesterol and impairs endothelial function in healthy men and women *Arterioscler. Thromb. Vasc. Biol.* **21** 1233–7
- Enderler M D, Schroeder S, Ossen R, Meisner C, Baumbach A, Haering H U, Karsch K R and Pfohl M 1998 Comparison of peripheral endothelial dysfunction and intimal media thickness in patients with suspected coronary artery disease *Heart* **80** 349–54
- Engelke K A, Halliwill J R, Proctor D N, Dietz N M and Joyner M J 1996 Contribution of nitric oxide and prostaglandins to reactive hyperemia in human forearm *J. Appl. Physiol.* **81** 1807–14
- Hardie K, Kinlay S, Hardy D, Wlodarczyk J, Silberberg J and Fletcher P 1997 Reproducibility of brachial ultrasonography and flow-mediated dilatation (FMD) for assessing endothelial function *Aust. NZJ. Med.* **27** 649–52
- Higashi Y, Sasaki S, Kurisu S, Yoshimizu A, Sasaki N, Matsuura H, Kajiyama G and Oshima T 1999 Regular aerobic exercise augments endothelium-dependent vascular relaxation in normotensive as well as hypertensive subjects: role of endothelium-derived nitric oxide *Circulation* **100** 1194–202
- Histand M B and Anliker M 1973 Influence of flow and pressure on wave propagation in the canine aorta *Circ. Res.* **32** 524–9
- Hosono M, Suehiro S, Shibata T, Sasaki Y, Kumano H and Kinoshita H 2000 Duplex scanning to assess radial artery suitability for coronary artery bypass grafting *Japan. J. Thorac. Cardiovasc. Surg.* **48** 217–21
- Joannides R, Haefeli W, Linder L, Richard V, Bakkali E, Thuillez C and Luscher T 1995 Nitric oxide is responsible for flow-dependent dilatation of human peripheral conduit arteries in vivo *Circulation* **91** 1314–9
- Landowne M 1957 A method using induced waves to study pressure propagation in human arteries *Circ. Res.* **5** 594–601
- Lieberman E H, Gerhard M D, Uehata A, Selwyn A P, Ganz P, Yeung A C and Creager M A 1996 Flow-induced vasodilation of the human brachial artery is impaired in patients <40 years of age with coronary artery disease *Am. J. Cardiol.* **78** 1210–4
- Maltz J and Budinger T 2003 Instrument for the noninvasive evaluation of human arterial endothelial function via measurement of changes in the transit time of an artificial pulse *Proc. 25th Annu. Int. Conf. IEEE Eng. Med. Biol. Soc.* **4** 3133–6
- Meredith I T, Currie K E, Anderson T J, Roddy M A, Ganz P and Creager M A 1996 Postischemic vasodilation in human forearm is dependent on endothelium-derived nitric oxide *Am. J. Physiol.* **270** H1435–40
- Mombouli J V and Vanhoutte P M 1999 Endothelial dysfunction: from physiology to therapy *J. Mol. Cell. Cardiol.* **31** 61–74
- Moore D F, Altarescu G, Pursley R, Campia U, Panza J A, Dimitriadis E and Schiffmann R 2002 Arterial wall properties and womersley flow in fabry disease *BMC Cardiovasc. Disord.* **2** 1
- Neunteufl T, Heher S, Katzenschlager R, Wolf G, Kostner K, Maurer G and Weidinger F 2000 Late prognostic value of flow-mediated dilation in the brachial artery of patients with chest pain *Am. J. Cardiol.* **86** 207–10
- Nichols W and O'Rourke M 1998 *McDonald's Blood Flow in Arteries* 4th edn (London: Edward Arnold)
- Ramsey M, Goodfellow J, Bellamy M, Lewis M and Henderson A 1996 Non-invasive detection of endothelial dysfunction *Lancet* **348** 128–9
- Sorensen K, Celermajer D, Spiegelhalter D, Georgakopoulos D, Robinson J, Thomas O and Deanfield J 1995 Non-invasive measurement of human endothelium dependent arterial responses: accuracy and reproducibility *Br. Heart J.* **74** 247–53
- Tagawa T, Imaizumi T, Endo T, Shihramoto M, Harasawa Y and Takeshihta A 1994 Role of nitric oxide in reactive hyperemia in human forearm vessels *Circulation* **90** 2285–90
- Takase B, Uehata A, Akima T, Nagai T, Nishioka T, Hamabe A, Satomura K, Ohsuzu F and Kurita A 1998 Endothelium-dependent flow-mediated vasodilation in coronary and brachial arteries in suspected coronary artery disease *Am. J. Cardiol.* **82** 1535–9
- Tanaka S, Gao S, Nogawa M and Yamakoshi K 2003 A new non-invasive device for measuring instantaneous blood pressure in radial artery based on the volume-compensation method *Proc. 25th Annu. Int. Conf. IEEE Engineering in Medicine and Biology Society* pp 3149–52

- Teragawa H, Kato M, Kurokawa J, Yamagata T, Matsuura H and Chayama K 2001 Usefulness of flow-mediated dilation of the brachial artery and/or the intima-media thickness of the carotid artery in predicting coronary narrowing in patients suspected of having coronary artery disease *Am. J. Cardiol.* **88** 1147–51
- Vita J, Treasure C, Nabel G, McLenachan J, Fish R, Yeung A, Vekshtein V, Selwyn A and Ganz P 1990 The coronary vasodilator response to acetylcholine relates to risk factors for coronary artery disease *Circulation* **81** 491–7
- Vuilleumier P, Decosterd D, Maillard M, Burnier M and Hayoz D 2002 Postischemic forearm skin reactive hyperemia is related to cardiovascular risk factors in a healthy female population *J. Hypertens.* **20** 1753–7
- Wascher T C, Bammer R, Stollberger R, Bahadori B, Wallner S and Toplak H 1998 Forearm composition contributes to differences in reactive hyperaemia between healthy men and women *Eur. J. Clin. Invest.* **28** 243–8
- Zeiger A, Drexler H, Wollschäger H and Just H 1991 Modulation of coronary vasodilator tone in humans: progressive endothelial dysfunction with different early stages of coronary atherosclerosis *Circulation* **83** 391–401