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Measurement of brachial artery endothelial function using a standard blood pressure cuff

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Abstract

The integrity of endothelial function in major arteries (EFMA) is a powerful independent predictor of heart attack and stroke. Existing ultrasound-based non-invasive assessment methods are technically challenging and suitable only for laboratory settings. EFMA, like blood pressure (BP), is both acutely and chronically affected by factors such as lifestyle and medication. Consequently, lab-based measurements cannot fully gauge the effects of medical interventions on EFMA. EFMA and BP have, arguably, comparable (but complementary) value in the assessment of cardiovascular health. Widespread deployment of EFMA assessment is thus a desirable clinical goal. To this end, we propose a device based on modifying the measurement protocol of a standard electronic sphygmomanometer.

Methods—The protocol involves inflating the cuff to sub-diastolic levels to enable recording of the pulse waveform before and after vasodilatory stimulus. The mechanical unloading of the arterial wall provided by the cuff amplifies the distension that occurs with each pulse, which is measured as a pressure variation in the cuff. We show that the height of the rising edge of each pulse is proportional to the change in lumen area between diastole and systole. This allows the effect of vasodilatory stimuli on the artery to be measured with high sensitivity. We compare the proposed cuff flow-mediated dilation (cFMD) method to ultrasound FMD (uFMD).

Results—We find significant correlation (r=0.55, p = 0.003, N=27) between cFMD- and uFMD-based metrics obtained when the release of a 5-minute cuff occlusion is employed to

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induce endothelial stimulus via reactive hyperemia. cFMD is approximately proportional to the square of uFMD, representing a typical increase in sensitivity to vasodilation of 300–600%.

Conclusion—This study illustrates the potential for an individual to conveniently measure his/her EFMA by using a low-cost reprogrammed home sphygmomanometer.

1. Introduction

There is overwhelming evidence that the endothelial function of major arteries is a sensitive and independent early predictor of both incipient atherogenesis [1, 2, 3, 4, 5, 6, 7] and future cardiovascular events such as heart attack and stroke [8, 9, 10]. Endothelial function in the brachial artery is strongly correlated with coronary endothelial function [11, 12, 7], and thousands of published studies have assessed systemic endothelial function via ultrasonography of the brachial artery [13]. In these endothelium-dependent flow-mediated dilation (FMD) studies, brachial artery diameter is measured before and after 5 minutes of flow occlusion [6]. When the occlusion is released, reactive hyperemia (RH) ensues. This increased blood flow activates shear stress sensors on the endothelial cells. In this way, the endothelium is stimulated to release factors that relax the surrounding vascular smooth muscle. In humans, nitric oxide (NO) is the predominant endothelium-derived relaxing factor, although others such as prostacyclin and endothelium-derived hyperpolarizing factors (EDHFs) also play a role [14]. The small diameter increase of 300–500 microns that constitutes the response in a healthy brachial artery is difficult to measure reliably using ultrasound imaging. Even tiny amounts of subject motion can sufficiently shift the position of the probe relative to the artery and thus introduce significant errors. Consequently, a great degree of technical experience and subject compliance is required to obtain high-quality measurements [15, 16, 17, 7].

Notwithstanding these difficulties, the value of brachial artery FMD assessment is very well supported by clinical data [7]. A meta-analysis of studies of close to 2500 patients found that brachial and coronary endothelial function have similar power to predict serious cardiovascular events [18]. More recently, a large (N=3026) multi-ethnic study of atherosclerosis (MESA) showed that FMD of the brachial artery independently predicts future cardiovascular events even after adjustment for Framingham Risk Score. When combined, FMD and Framingham risk constitute a more powerful predictor than either metric alone [10].

The clinical value of endothelial function measurement, as established by the past 25 years of research, is reviewed in [7].

Since endothelial function is both acutely and chronically affected by lifestyle factors that influence cardiovascular disease (CVD) risk, endothelial function measurements are useful for monitoring response to medication, dietary changes and exercise regimens.

It is important to note that endothelial dysfunction may be evident far in advance of atherosclerotic pathology, such as in children as young as 8 years of age [19, 20].

The fact that endothelial function is sensitive to many factors is both an advantage and a disadvantage. To obtain an estimate of the endothelial function that is independent of acute influences requires careful control over such factors [21]. However, sensitivity to acute changes in endothelial response can enable individuals to obtain feedback regarding the effects of behavior (e.g., smoking) on arterial function. This does, however, require a measurement device such as the one we describe here, which can easily obtain measurements and thus make possible more frequent observations.

A more convenient and accurate method of endothelial function assessment would also allow clinicians and individuals to obtain sensitive feedback regarding the effect on arterial function of interventions such as smoking cessation, diet and exercise regimens, antihypertensive therapy, and cholesterol-lowering medications on arterial function [22, 23, 24, 7].

The recognized clinical value of endothelial function measurements, and the technical difficulties associated with ultrasonic FMD measurement (uFMD), spurred the development of the two FDA-approved products that are currently commercially available for endothelial function testing in the United States. Both are based on measurements obtained in peripheral resistance vessel beds rather than in conduit arteries.

The Endo-PAT2000 system from Itamar Medical (Caesarea, Israel) analyzes the pulse amplitude in a finger before and after application of endothelial stimulus. While at least 46% of the observed changes in pulse amplitude are blocked by NO synthase (NOS) inhibitors, mechanisms other than those mediated by NO are likely to contribute towards the measured response [25]. This is most probably a consequence of the different mechanisms involved in endothelium-dependent, flow-mediated arterial and arteriolar/microvascular vasodilation. Inhibition of endothelial nitric oxide synthase [eNOS] in major arteries largely abolishes FMD [26]). This is not true of smaller arteries, in which EDHFs play an increasingly greater role relative to NO as arterial caliber decreases [14].

Two large (> 1800 subject) cross-sectional studies found an association between EndoPAT measurements and accepted cardiovascular risk factors [27, 28]. However, the correlations between EndoPAT and FMD were low in the respective studies: r=0.094 (N=1843), and r=0.19 (N=5000). Correlations decreased further when adjusted for age and sex. This is likely due to different physiological bases of the measurements [7]. Some of the results of these studies suggest the influence of potentially serious confounding factors. For example, while it is well known that endothelial response tends to decrease with age, older subjects exhibited better endothelial response, as measured using Endo-PAT in some studies.

From the point of view of monitoring the effects of interventions aimed at improving endothelial function, the collective body of evidence suggests monitoring arterial endothelial function is more important than assessing resistance vessel response [7]. Smoking, for example, causes dysfunction of coronary artery endothelial function but does not affect microvascular endothelial function [29]. This suggests monitoring the effects of smoking cessation would require a metric of endothelial function in major arteries.

The second approved device is the VENDYS system developed by Endothelix, Inc. of Houston Texas. This system measures the cutaneous reactive hyperemic response using hand skin temperature measurement during 2 minutes of brachial artery occlusion and ensuing RH. During occlusion, skin temperature drops in the distal hand. As blood flow is restored, the temperature increases. Studies indicate that the recovery of skin temperature is slowed in subjects having higher Framingham risk scores and other metrics of CVD and CVD risk. Interestingly, substantial temperature changes are also observed in the contralateral hand that experiences no reactive hyperemic episode [30]. This suggests significant neural involvement in the response. For this reason and the results of Wong et al. [31] it is reasonable to predict that this response is not a measure that is principally dependent on NO release and would not thus be expected to be blocked by NOS inhibitors.

We previously developed an instrument for arterial endothelial function assessment, the “relaxoscope,” that uses changes in the transit times of artificial pulses (mechanically induced in the radial artery) to measure the effects of vasorelaxatory stimuli on radial artery tone. This device demonstrates greater sensitivity (137%) to vasorelaxation, lower between-measurement coefficient-of-variation, and improved ease-of-use, relative to uFMD. However, a skilled operator is still required to position the pulse induction device and ultrasound probe [32].

Here, we present a method of measuring changes in the cross-sectional area of the brachial artery that requires neither relatively costly and bulky ultrasound equipment, nor any technical skill on the part of the operator. Instead of ultrasound, a standard blood pressure cuff is used to take the measurement. The cuff is partially inflated during the measurement process, so that changes in the area and compliance of the vessel can be calculated from very small pressure variations in the cuff. The partially inflated cuff removes (mechanically unloads) stress from the arterial wall, and this amplifies the absolute change in area and compliance seen in response to endothelial stimulus [33], allowing ensuing vasorelaxation to be measured much more easily. The same cuff may be used to occlude the artery and thus provide reactive hyperemic stimulus for FMD measurements.

The next section explains the physical and physiological basis of the measurement. We then describe the initial prototype of the device, and then demonstrate that the device may be realized by reprogramming a consumer-oriented electronic sphygmomanometer. The method is then evaluated on human volunteers and the results are compared to ultrasound-based FMD (uFMD) studies performed on the same limb 10 minutes following the cuff FMD (cFMD) measurements.

A list of abbreviations used in this paper appears in Table 1.

2. Principles of operation

The key to making FMD much easier to assess is to use a cuff to measure changes in arterial cross-sectional area from volume changes reflected in blood pressure cuff measurements, instead of using ultrasound imaging to measure arterial diameter. This allows us to eventually create a subject-operated consumer-oriented measurement device that can take

advantage of convenient hardware and software platforms, such as smart phones and tablets, as we will describe in Section 5.2.

When the cuff is partially inflated so that it fits the arm snugly, changes in cuff pressure are proportional to changes in the volume of the underlying arm (this is the basic principle of plethysmography). Since blood volume changes most rapidly in the conduit arteries, the rising edge of each pulse (diastole to systole) reflects changes in the volume of these arteries enclosed by the cuff.

The induction of local reactive hyperemia by means of cuff occlusion and subsequent release does not change systemic blood pressure. Under these circumstances (which should ideally be verified for each study), the pressure changes observed from diastole to systole are proportional to the concomitant volume changes. Let ΔV_b and ΔV_r denote the volume changes from diastole to systole under baseline and post-stimulus response conditions. Since the cuff is part of a sealed pneumatic system, the pressure-volume product is constant ($PV = k$). If the cuff snugly encloses the limb and the outer cuff sheath is non-elastic, the total volume (the volume of the enclosed limb + the volume of the cuff) maintains a constant value even as the blood volume changes. An increment in arterial pressure leads to an increase in arterial volume, which reduces the volume of the cuff by an equal amount (by compressing its contents). This in turn effects a pressure increase in the cuff that is proportional to the volume change in the artery.

Stating this formally:

$$V_l + V_c = V_{\text{total}} = (V_l - \Delta V) + (V_c + \Delta V) \quad \text{and}$$

$$P_c V_c = k = (P_c + \Delta P)(V_c + \Delta V),$$

where P_c is the cuff pressure, V_c is the cuff volume and ΔV is the change in volume of the enclosed limb, V_l. We now solve for the observed change in cuff pressure ΔP as:

$$\Delta P = -\frac{P_c}{V_c - \Delta V}\Delta V. \quad (1)$$

This is non-linear in ΔV, but since we have $\Delta V \ll V_c$ (the perturbation in the cuff volume due to the pulse is much smaller than the cuff volume), this strongly approximates a linear relationship with a slope $-P_c/V_c$. Since the length of the artery under the cuff, l, does not change appreciably during the cardiac cycle, we may thus assume that $\Delta P \propto \Delta A$, where A is the cross-sectional area of the arterial lumen. If we denote the pre- and post-stimulus areas as $A_b = V_b/l$ and $A_r = V_r/l$, respectively, the cFMD metric is given by:

$$\text{cFMD}\% = \left[\frac{A_r}{A_b} - 1\right] \times 100. \quad (2)$$

This expression is an area analog of the standard FMD metric:

$$\mathrm{uFMD}\% = \left[\frac{d_r}{d_b} - 1\right] \times 100, \quad (3)$$

where d represents arterial diameter. It is important to remember that that the areas are obtained during wall unloading, and are not, in general, equal to $\pi d^2/4$ (under the assumption of a circular cross section), since those diameters are measured at full transmural pressure.

The small volume changes that occur in the artery lead to very small pressure changes in the cuff, which are difficult to measure accurately. However, as the degree of cuff inflation increases and more pressure is applied to the limb, mechanical stress on the wall of the artery is relieved by the cuff. This mechanical unloading decreases the influence of stiff collagen fibers on the vessel wall properties, and this leads to a large increase in vessel distensibility [34].

Figure 1 illustrates diametric distension waveforms obtained using M-mode wall tracking (Wall Track System II, Pie Medical, Maastricht, Netherlands). Decreasing the transmural pressure by 80 mmHg leads to a more than twenty-fold increase in maximum distension in response to the same diastolic to systolic pressure transition. This is consistent with the very carefully executed intra-arterial ultrasound measurements of Bank and co-workers [33]. Figure 2 illustrates the results of those studies, showing the change in brachial artery compliance across the full range of transmural pressure. The compliance characteristic is shown before and after the arterial smooth muscle is relaxed using nitroglycerin (NG). When the transmural pressure is reduced to ≈ 25 mmHg, we see that the absolute difference in vessel compliance between the baseline and relaxed state is maximized. The relevant observation is that relaxation of the artery (such as that due to FMD) is much easier to measure when the artery wall is unloaded, simply because the magnitude of the induced change is a larger quantity. A larger change in compliance means that a larger increase in arterial cross-sectional area is achieved for a given pressure rise from diastole to systole.

In the above theoretical justification of the proposed measurement method, we assume that the tissue between the cuff and artery is incompressible, and that it does not change in volume between the pre- and post-stimulus intervals. The thickness and consistency of this tissue will affect the absolute relationship between the volume of the artery and the pressure in the cuff. However, since the cFMD metric is normalized to a baseline measurement, as long as this relationship does not change between the pre- and post-stimulus measurement intervals, the characteristics of this tissue should not influence the results.

It is reasonable to expect that the vasodilatory stimulus will cause some vasodilation of resistance vessels in the surrounding tissue, and elsewhere in the limb distal to the occlusion [36, pp. 258–9]. The former effect will cause the cFMD metric to somewhat overestimate the pure arterial response. The effect of the latter is to decrease wave reflection at distal sites (owing to arteriolar dilation), and this may reduce the amplitude of the systolic peak, leading to underestimation of the arterial dilation. Since the rising edge of the distension waveform

(luminal volume) is in phase with the pressure waveform [37], changes in wave reflection in the distal limb will bias both uFMD and cFMD to a similar extent. We consequently can ignore this effect as a differential confounding influence.

To quantify the effect of vasodilation in intervening tissues, we compare the 5%–95% rise times of the distension waveform (obtained using M-mode wall tracking, as was used to produce the waveforms in Figure 1) with the cuff pressure waveform. Similar rise times would imply that this part of the cuff pressure waveform (from which the cFMD metric is chiefly derived) represents the direct effect of arterial luminal area increase. The reason for this is that low caliber vessels provide much larger resistance to flow than conduit vessels and thus the time constant for volume change in these vessels is much longer. For example, in the human finger, the pulse transit time over the short distance from the digital arteries to the skin of the same finger is more than 200 ms, which is longer than the rise times of both the distension and cuff pressure waveforms [38]. As an illustrative example, we examined 55 typical rising edges of the acquired cuff pulse pressure waveform and calculated a mean (± SD) rise time of 133±8 ms. The corresponding distension mean rise time is 122±2 ms. Since the thickness of the intervening tissue bed is much larger than that encountered in the finger, it is unlikely that the volume change in the resistance bed could appreciably contribute to the rising edge of the waveform, since the volume increase in the tissues would occur only after we have made our cFMD measurement for a particular pulse. We thus believe that the cFMD metric is chiefly affected by dilation of the artery rather than smaller resistance vessels.

3. Methods

3.1. Study protocol

A typical study proceeds as follows:

i. With the subject seated or supine, the cuff is placed around the upper arm.

ii. Blood pressure is measured.

iii. The cuff is inflated to a value P_m, termed the measurement pressure, which must be less than the mean arterial pressure, for a period T_m = 30 s. During this time interval, we measure and record the pressure fluctuations in the cuff. These data constitute a pre-stimulus baseline measurement.

iv. The cuff is deflated.

One or more baseline measurement series are now obtained. To acquire N_b baseline series, Steps (iii)–(iv) are repeated, with a waiting period of T_w=30 s between inflations. These rest periods allow restoration of venous return. Typically, we set N_b = 3 in our studies.

v. The stimulus is applied. This is either 3–5 min of cuff occlusion to suprasystolic pressure P_s (for studies of endothelial function) or a dose of sublingual NG (for studies of endothelium-independent vasodilation).

vi. After T_p=45 s have elapsed following cuff release or drug administration, a series of up to N_r=10 repeat measurement intervals ensue. In each interval, the

cuff is inflated to P_m for T_m seconds, after which it is deflated for T_w seconds. This large number of repeat measurements (N_r) is required only when one wishes to record the return of the vessel toward baseline.

vii. Blood pressure is measured again to ensure it has not changed appreciably since step (ii).

viii. Each post-stimulus response is then compared to the average baseline response, to yield the area-based cFMD metric (Equation 2) defined above. As is the objective in uFMD studies, we seek the value of maximal vasodilation within the response time course as a fraction of the baseline condition of the artery.

It is very important to ensure that P_m remains below the diastolic pressure throughout the entire study. Should P_m exceed the diastolic pressure, the artery will collapse during at least part of the cardiac cycle. This "clipping" of the pressure waveform will generally reduce the measured ΔP for each pulse. Since any subsequent increases in area change will then be only partially reflected in the measurements, the quantity A_t/A_b may be underestimated.

In our studies, we set P_m to be 10 mmHg less than the diastolic pressure measured in Step ii above. With reference to Figure 2, in this region of the compliance versus transmural pressure characteristic, the ratio of the curves of dilated and baseline states is relatively insensitive to small changes (±5 mmHg) in transmural pressure. This reduces the sensitivity of the measurement to the value of P_m used, and to errors in the diastolic blood pressure measurement.

Steps (ii) through (viii) are completely automated and ensue without the need for user intervention.

3.2. Device prototypes

Three prototypes were developed to implement the protocol described above in a completely automated fashion. The basic requirement is a device that can maintain a substantially constant pressure in the cuff, while measuring pressure changes with a resolution of approximately 0.1 mmHg. The time constant of pressure regulation needs to be sufficiently long so as not to cancel the pulse signal. The three prototypes are described in detail in the Appendix.

3.3. Signal processing

For the T_m second time record for measurement series i, $p(t)$ is processed as follows:

i. A 2-pole high-pass Butterworth filter with cutoff frequency of 0.5 Hz is applied to remove the DC component of the cuff pressure signal, yielding the AC signal, $p_{AC}(t)$,

ii. A peak and foot detection algorithm identifies the individual pulses. Outliers in terms of pulse height, rise time, and period are discarded.

iii. For systems implemented with continuous pressure control, the remaining pulse heights are averaged to yield a value $\overline{\Delta P_i}$ for each measurement interval.

For devices that use on-off control to regulate pressure, linear regression is used to adjust the pulse heights to the mean cuff pressure over all intervals *i*. The mean of the adjusted pulse heights for each *i* is then taken. This reduces bias introduced by variations in unloading pressure that occur during each measurement interval when on-off control is employed. These biases are introduced by shifting the operating point along the transmural pressure axis of Figure 2. Based on the behavior of these curves, it appears reasonable to fit a linear model around an operating point close to 20 mmHg transmural pressure.

iv. Finally, the maximum of the cFMD metric in Equation 2, analogous to that used for uFMD, expressed directly as a function of the measurement data, is calculated as:

$$\mathrm{cFMD}_{\max}\%=\left[\frac{\max_{N_r\geq k>N_b}\overline{\Delta P}_k}{1/N_b\sum_{n=1}^{N_b}\overline{\Delta P}_n}-1\right]\times 100 \quad (4)$$

and reported to the user. This value reflects the ratio between the mean of all baseline measurement set means and the highest mean among the post-stimulus measurement intervals. Where this metric applies to general stimulus (e.g., reactive hyperemia or nitroglycerin), we denote it $\mathrm{cD}_{\max}\%$.

3.4. Evaluation in human subjects: preliminary studies

We seek first to establish whether the method:

i. Is sensitive to smooth muscle relaxation due to sublingual nitroglycerin.

ii. Is sensitive to vasodilation following reactive hyperemia in subjects with very low CVD risk.

iii. Exhibits good repeatability,

Since the day-to-day FMD response is dependent on many factors (e.g., food, medication, menstrual state and time-of-day), the consistency of the measurement method itself is best assessed via nitroglycerin studies.

A total of three subjects are examined up to six times each for each of three stimuli:

i. RH following 5 minutes of cuff occlusion (RH5).

ii. 400 μg of sublingual nitroglycerin (NG).

iii. No stimulus (NS), equivalent to no cuff inflation, or zero dose of drug.

Table 2 provides details of the three subjects examined and the number of repeat tests performed for each stimulus for a total of 26 experiments. These subjects were examined at Lawrence Berkeley National Laboratory under an approved human subjects protocol.

Prototype I, as well as an earlier prototype that was based on a continuously regulated pressure source, were employed for these studies.

3.5. Evaluation in human subjects: correlation between cFMD and uFMD

While our small-sample preliminary studies can potentially provide evidence of the sensitivity and repeatability of the method, more convincing validation requires an adequately powered comparison of cFMD with an accepted measure of FMD. We do this by comparing cFMD and uFMD methods in the same subjects on the same day and at the same time of day. We now describe the experimental design of this study.

3.5.1. Study population—We examined human volunteers currently involved in a study of the effects of omega-3 fatty acid supplementation on vascular physiological parameters in patients with peripheral artery disease (PAD). These volunteers consisted of subjects with known PAD and aged-matched, non-PAD controls. Most of the controls, however, were of advanced age and had other cardiovascular disease. This population was chosen for convenience and availability: inclusion of controls with a lower risk of CVD would enable evaluation of the correlation between cFMD and uFMD over a wider range of endothelial competency. Since uFMD has high variability, it is difficult to differentiate poor responders into multiple tiers. The scatter of uFMD measurements alone can mask correlations for such groups. We proceeded with the study notwithstanding this anticipated difficulty.

The characteristics of the subjects who participated in this study are listed in Table 3. These subjects were examined at the San Francisco VA Medical Center, under approval from the relevant ethics board.

3.5.2. Ultrasound FMD study protocol—uFMD measurements are performed in accordance with currently recommended guidelines and standards [21, 39] and as we describe in [40]. Before the study, subjects are required to fast for at least 8 hours and desist from nicotine products for at least 4 hours. A history of recent medications is recorded. Subjects rest for 10 minutes in a supine position in a darkened room at 23°C. The subject's arm is then extended onto a movement-constraining pillow with the palmar aspect oriented anteriorly. A 5-cm-wide tourniquet blood pressure cuff is placed on the upper arm distal to the insertion of the deltoid. The length of the brachial artery is surveyed using B-mode ultrasound (Philips HD11, Philips Healthcare, Best, Netherlands) with a broadband linear array transducer with a 3–12 MHz range (Philips L12-3) until a straight segment with a visible registration structure can be located. The probe is oriented so that the artery is at least 3 cm below the surface of the skin, and the focus is aligned with the deep boundary of the vessel. The protocol requires that the boundary between the intima and lumen be clearly visible. Prior to cuff inflation, the baseline diameter of the vessel and blood-flow velocity are recorded for 60 seconds using electrocardiogram-gated image capture software (Brachial Imager, Medical Imaging Applications LLC, Coralville, IA). Baseline blood-flow velocity is recorded for 60 s using an insonation angle of 60°. The Doppler sample gate is positioned to cover the center, but not the edges, of the lumen. The probe remains in a fixed position between measurements. The blood pressure cuff is then inflated to the greater of 250 mmHg or 50 mmHg above the subject's systolic blood pressure for a period of 5 minutes. Recording of the B-mode images begins 10 s prior to cuff release. Blood-flow velocity is assessed for a period of 30 seconds post-cuff release using the methods described above. B-

mode images are recorded until 3 minutes post-cuff release. Analysis of the images is performed using continuous edge-detection software (Brachial Analyzer, Medical Imaging Applications LLC). Baseline diameter is recorded as the mean of 60 seconds of data. From recordings obtained during the reactive hyperemic phase, the exact moment of cuff release is determined. Hyperemia diameter is calculated using a pre-determined time window (55–65 s post-cuff release). uFMD% is calculated as:

$$\mathrm{uFMD\%}=100\times\frac{d_{60\mathrm{S}}-\overline{d}_{\mathrm{b}}}{\overline{d}_{\mathrm{b}}}, \quad (5)$$

where d_{60}s represents the diameter measured at 60 s after cuff release, and $\bar{d}_b$ is the average baseline diameter.

3.5.3. Sample size selection—We base our sample size on that recommended for uFMD, since our preliminary data suggest that the cFMD method is less variable and much more sensitive than uFMD.

Sample sizes of 20–30 per group have been previously used in uFMD studies that attempt to compare endothelial function between two groups [21]. With this sample size, the minimal statistically significant change that can be detected with an intervention at this group size is an absolute change in FMD of 1.5% to 2% (α=0.05, β=0.2 [power of 80%]).

The statistics obtained from 399 papers that appear in the meta-analysis of [13] are also useful for sample size selection. It is reasonable to expect that the measurement variance for a meta-analysis is higher than that for individual laboratories and will consequently lead to an overestimate of the number of subjects required. Power analysis using the G*POWER 3.03 software package [41] for a power of 80% at a confidence level of 95% yields a sample size of 21 subjects per group to differentiate subjects in the 1st and 3rd tertiles of Framingham risk, and 63 per group to differentiate between the 1st and 2nd tertiles. Based on the literature cited above, we choose a minimum group size of 21.

Since the purpose of this part of the study is to determine whether cFMD and uFMD are correlates, rather than investigate FMD under different disease states, we combine the data from control and PAD subjects in one group.

3.5.4. cFMD measurement apparatus—Prototype II was employed for these studies, owing to its compact form factor, ease of use, and availability at the time these experiments were conducted.

3.6. Statistical methods

For the experiments described in Section 3.4, we use a one-tailed Student t-test to determine whether to accept the null hypothesis that the endothelial and smooth muscle stimuli do not increase the measured responses $\mathrm{cFMD}_{\mathrm{max}}$ to RH, and $\mathrm{cD}_{\mathrm{max}}$ to NG, respectively.

To analyze the results of the experiments comparing cFMD with uFMD in Section 3.5, we prepare a scatter plot of the two measurements. In this case, where we find a substantially linear relationship between $uFMD_{max}$ and $cFMD_{max}$ over the range of sample values, we calculate the Pearson product-moment correlation coefficient r and perform linear regression on the data points. Our confidence in r is based on the fact that the quantity $t = r / \sqrt{(1-r)^2/(N-2)}$ follows Student's t-distribution with $(N-2)$ degrees-of-freedom [42, p. 431] for $N \geq 6$. Here, N is the number of data point-pairs (human subjects examined once each) used to calculate r.

While basic physical arguments suggest cFMD should be proportional to the square of uFMD, the elastic operating point of the vessel differs between the two measurements, so a more complicated unknown non-linear relationship generally applies. A linear model, while not ideal in such cases, can be useful for correlation analysis of samples of what we hypothesize are both monotonic functions that are proportional to the vessel response to stimulus.

The objective of our correlation analysis is to compare uFMD to cFMD regardless of subject membership in the control or PAD groups. Comparison of endothelial function between control and PAD groups is well documented [43, 44] and was not the primary purpose of this study. Nevertheless, in a sensitivity analysis, we determine whether uFMD and cFMD are significantly worse in the PAD group relative to controls, using the one-tailed Student's t-test.

In both sets of experiments, p-values less than 0.05 are considered statistically significant.

To ensure the applicability of the above methods, we tested the uFMD and cFMD measurements for normality using a single-sample Kolmogorov-Smirnov test, with the significance level set at 1% [45].

4. Results

4.1. Preliminary studies of cFMD

In Table 4 we calculate the maximum response for each stimulus and evaluate the statistical significance of the change relative to the no stimulus (NS) case.

4.2. Flow-mediated dilation: Ultrasound- versus cuff-based measurements

Figure 4 is a scatter plot that shows cFMD vs uFMD measurements for N=27 subjects. The slope of the regression line indicates that cFMD is 346% more sensitive to the underlying stimulus than uFMD.

When systolic hypertensive subjects (those having systolic blood pressure greater than 140 mmHg) are removed from the dataset, we find an increased correlation, as shown in Figure 5. (The rationale behind performing this particular analysis is based on the correlation between arterial stiffness and endothelial dysfunction observed in [46]. The relevance of those results to the present study is discussed in Section 5 below.)

For uFMD, the PAD group responses were 31% lower than those of the control groups (p=0.04). The PAD group exhibited 24% lower cFMD than controls (p=0.03).

Both the uFMD and cFMD measurements pass the Kolmogorov-Smirnov test for normality at a 1% significance level.

5. Discussion

A prudent first step in the evaluation of any new method or protocol for assessment of endothelial function is to establish sensitivity to endothelium-independent smooth muscle relaxation. By comparing the response of subjects to 400 μg and a zero dose of sublingual NG (no stimulus [NS]), we can establish whether the method is sensitive to the smooth muscle relaxation that is the effect of endothelial stimulus. Smooth muscle relaxation and vasodilation are the end results of NO stimulus regardless of whether NO is endogenously generated or exogenously supplied.

The data shown in Figure 3 demonstrate with great statistical certainty that the proposed metric can detect changes due to NG vs. NS (+70%, $p = 6.25 \times 10^{-6}$). Not only do the distributions for NG and NS responses differ, but there is in fact no overlap of the distributions of these data within the time interval of maximum response, spanning from 5 minutes to 15 minutes after the administration of the drug. We have previously determined that NG at this dose does not produce changes in systemic blood pressure that could confound these measurements [32]. This is especially important in the case of the present method, as correct operation according to the arguments provided in Section 2 requires that blood pressure remain constant between baseline and post-stimulus measurement intervals.

Since a 400 μg sublingual dose of NG is reported to elicit maximal smooth muscle dilation [47, 48], the next step is to determine whether RH following 5 minutes (RH5) of cuff occlusion produces a measurable change in the metric in individuals expected to have sound endothelial function.

RH5 indeed produces a significant change vs NS (+51%, $p = 1.19 \times 10^{-5}$). In the 4 minutes following cuff release, there is no overlap between the RH5 and NS distributions (during the window of maximum response) evident in Figure 3.

Table 4 summarizes the above findings.

These preliminary studies confirm that the method is sensitive to vasorelaxatory stimuli, but comparison with an established method is needed to determine whether a proportional relationship exists between the proposed and accepted metrics of endothelial function. Figure 4 displays a scatterplot of measurements from the established method of uFMD and cuff FMD. We regard the correlation of $r = 0.55$ observed in the data depicted in Figure 4 as moderate to strong, in view of the fact that our study population has substantially poorer uFMD than would be expected of a general population, and since our sample size limits us to differentiation of the first and third tertiles of uFMD response. Our population sample was a convenience sample, with an over-representation of individuals with cardiac risk factors. The uFMD responses that we observed in this study are typical of the first and second

tertiles of endothelial response for a larger sample of the general population. We are thus not exploring the full natural “dynamic range” of FMD and this makes it more difficult to observe stronger correlations.

A potential limitation of this study is that, while we examined both control subjects and PAD patients, we did not compare responses in these subgroups. Our objective was to determine the correlation between cFMD and uFMD, regardless of the subgroup membership of the individuals. Endothelial function, measured either in terms of uFMD or cFMD, is expected to be impaired in PAD patients, given the pathophysiology of PAD [43, 44]. Gross focal atherosclerotic disease, which might confound assessment of global endothelial function by both uFMD and cFMD, was not apparent in the B-mode ultrasound images of the measurement sites in any of these studies. It is reassuring that in the PAD patients we examined, the impairments of uFMD and cFMD, relative to controls, were similar (31% and 24% respectively).

Subjects with isolated systolic hypertension have been found to exhibit both high aortic pulse wave velocity (arterial stiffness) and impaired FMD [46]. We thus performed a sub-group analysis excluding subjects with systolic pressures above 140 mmHg, and found that the correlation between cFMD and uFMD increases to 0.82 ($p < 0.0002$), as shown in Figure 5. It is possible that mechanical unloading of stiff arteries allows more flow-mediated dilation to occur, since such arteries may not be as severely restricted by their collagen framework when the wall is under less stress. (Models fit to in vivo measurements indicate that collagen fibers that act in parallel with the smooth muscle are increasingly recruited as transmural pressure rises [34].) If this is the case, uFMD may be systematically underestimating FMD in these subjects. This contention is further supported by reported correlations between endothelium-dependent and endotheium-independent dilations (EDD and EID) [48]. In this large study of 800 subjects, Adams et al. found a correlation of 0.41 between EDD and EID. When those subjects at higher risk of atherosclerosis were removed (diabetics as well as those with a history of tobacco smoking), the correlation coefficient fell to 0.24. It is quite possible that the impaired dilation attributed to “smooth muscle dysfunction” [48] is in fact due to an impaired ability of the vessel to dilate even when the smooth muscle is relaxed. It would be interesting to conduct a similar study to compare EDD and EID in the presence of mechanical unloading. Such studies may be conducted by measuring uFMD through a water-filled cuff. It is also important to confirm this finding by performing prospective studies designed to validate this particular hypothesis on the sub-group.

Alternatively, if cFMD is overestimating dilation, the cFMD metric may need to be calibrated to systolic blood pressure in order to remove bias that may occur in cases of subjects with systolic hypertension. Our current investigations are focused on understanding this phenomenon and developing model-based calibration.

Our results show that the sensitivity of the method to vasodilation is between three and six times greater than that of ultrasound-based imaging of arterial diameter in response to both flow-mediated dilation and NG. Most of this sensitivity increase owes to our measurement of area rather than diameter. As is often the case, a greater fundamental sensitivity to the

measured quantity makes it possible to use a simpler and lower-cost measurement system. We have realized the measurement in a device that is currently marketed to the consumer at a price of $99.

In concordance with current recommendations [39], we believe measurements of endothelial function in major arteries should ideally be based on NO-mediated FMD. In this sense, a limitation of the studies we perform here is that a single cuff is used for both measurement and occlusion. To assure that the dilation is purely NO-mediated requires a second cuff distal to the measurement cuff. This is equivalent to the case of wrist-occlusion in [49], where eNOS inhibition abolishes, rather than merely attenuates, FMD. The occlusion is then effected such that the measured segment of the artery is not subject to an ischemic stimulus during the occlusion interval. It is straightforward to modify the proposed method and apparatus to realize a split- or separate-cuff design. The combination of evidence and physical arguments presented here suggests that cFMD and uFMD will remain correlated regardless of the method of stimulus used.

While we have demonstrated that endothelial function may be assessed using equipment of the same complexity as that used for blood pressure measurement, the time taken to acquire the data is considerably longer. The minimum time needed for a study is envisaged as equal to: baseline measurement time (15 s) + post-measurement recovery time (30 s) + occlusion time + post-cuff-release time (60 s) + response measurement time (15 s) = 120 s + occlusion time. The only obvious way to shorten the study duration is to reduce the occlusion time. Corretti et al. compared uFMD responses elicited by upper arm (proximal) occlusion times of 1, 3 and 5 minutes [50]. Statistically significant responses were observed only in the case of 5-minute occlusions. While the mean dilations for 1- and 3-minute occlusions were substantial (respectively 2.1% and 7.8% vs 12.6% for 5-minute occlusion), the data were extremely variable. There is the possibility that owing to the sensitivity advantages of cFMD, measurements of the effects of a shorter occlusion might exhibit lower coefficients-of-variation. A 3-minute occlusion would allow measurement of cFMD in 5 minutes, which is attractive in comparison to conventional protocols. Whether shortening the occlusion interval changes the physiological basis of the observed response would need to be assessed via methods such as eNOS inhibition.

We believe the mass availability of a device for routine endothelial function assessment would prove clinically significant, since measurement of both acute and chronic changes in endothelial function could be accomplished for the first time. There are compelling reasons to believe that knowledge of acute variation in endothelial function in an individual is important. Since NO released by the endothelium is a potent inhibitor of the adhesion of platelets and leukocytes to the endothelial cell surface, and since adhesion of these cells is widely believed to be a necessary initiating event in atherogenesis [17], it is reasonable to infer that the proportion of time that the endothelium is dysfunctional constitutes an important indicator of disease risk. Just as dieters use a scale to measure body mass, and hypertensives use a home blood pressure monitor, portable endothelial function monitors may provide individuals with feedback regarding the impact of their lifestyle and medications on arterial health. Studies of larger and more diverse subject populations will be required to determine whether the methods proposed in this paper can address this desirable

clinical goal. In future work we intend to determine whether longitudinal series of endothelial function measurements can be used in conjunction with other metrics to predict impending cardiovascular events.

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Appendix

The following two prototypes implement the cFMD measurement method. Prototype I is a basic laboratory prototype, while Prototype II is implemented in a consumer blood pressure measurement device and is suitabile for routine and home use.

5.1. Prototype I

Figure 6 is a schematic of the first prototype, which uses an on-off pressure control system to maintain measurement and occlusion pressures. Inflation and deflation of the cuff are effected using a miniature diaphragm pump and solenoid valve (respectively, E161-11-050 and V2-20-5-PV-5-P88, Parker Hannifin Corp, Cleveland, OH). A semiconductor pressure sensor (NPC-1210, GE Novasensor, Fremont, CA) measures the pressure in the cuff for the purposes of regulation, as well as measurement of the pulse waveform.

A script running on the laptop fully automates the measurement protocol. To modify the cuff pressure, the script sets a pressure-calibrated voltage on a 12-bit digital-to-analog converter on the data acquisition card. A microcontroller (PIC12F675, MicroChip Technology, Inc., Chandler AZ) compares this voltage to the output voltage of the pressure sensor, and it actuates the pump and valve to maintain the desired pressure within a specified tolerance.

A disadvantage of using an on-off control algorithm is that pressure tends to decrease during a measurement owing to displacement of the arm tissue under the cuff. Frequent actuation of the pump to top-up air in the cuff introduces artifacts into the acquired pulse waveform. In the description of Prototype II below, we show how the acquisition may be modified to address this issue. Section 3.3 explains an alternative post-hoc approach based on regression analysis.

5.2. Prototype II

A consumer-oriented electronic sphygmomanometer (Wireless Blood Pressure Monitor, iHealth Lab Inc., Mountain View, CA) was modified by the manufacturer, under the supervision of our group, to implement the protocol described in Section 3.1. The device operates in the same manner as Prototype I.

The protocol parameters are set, and measurements are invoked, by a custom application (app) for Apple iOS handheld devices, including iPhone and iPad (Apple Inc., Cupertino, CA). Figure 7 shows the wireless cuff and the running app.

As shown in Figure 8, a measurement interval of length T is divided into two segments, T_1 and T_2, such that $T = T_1 + T_2$. The purpose of T_1 is to stabilize the pressure close to the measurement pressure set-point during the period when tissue compression under the cuff leads to a natural pressure drop. Once the pressure has stabilized, T_2 begins, during which no control of the pressure is exercised, or the criteria for initiating pressure corrections are considerably relaxed.

Different pressure tolerances ΔP_1 and ΔP_2 may be applied to the respective time segments T_1 and T_2. During interval T_n, adjustment of the pressure is only initiated when the cuff pressure $P < P_s - \Delta P_n$ or $P > P_s + \Delta P_n$. By setting, for example, $\Delta P_2 > \Delta P_1$, it is possible to avoid unnecessary servoing during T_2 that may render measurement data unusable. Figure 8 provides an example of the specification of these ranges and the interpretation of these quantities.

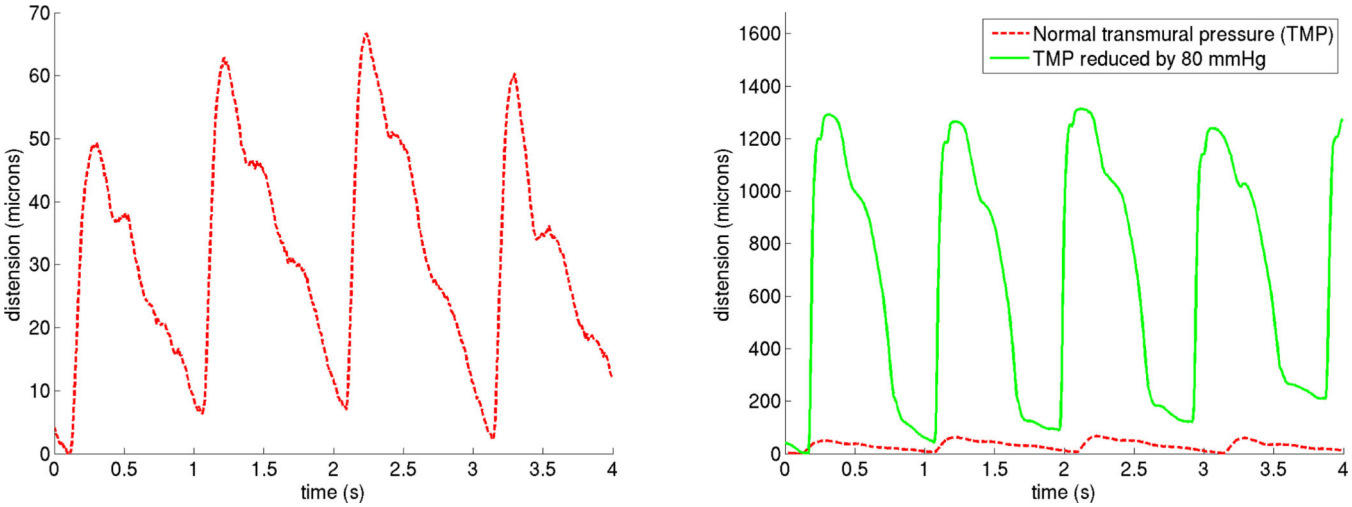

Figure 1.

Distension of the human brachial artery recorded by M-mode wall tracking. The subject is a 35-year-old male. *Left:* Distension waveform under normal conditions. *Right:* When the transmural pressure is decreased by 80 mmHg using an external cuff, the maximum distension of the artery increases more than twenty-fold over baseline conditions. (Note the change in scale for the ordinate axis.)

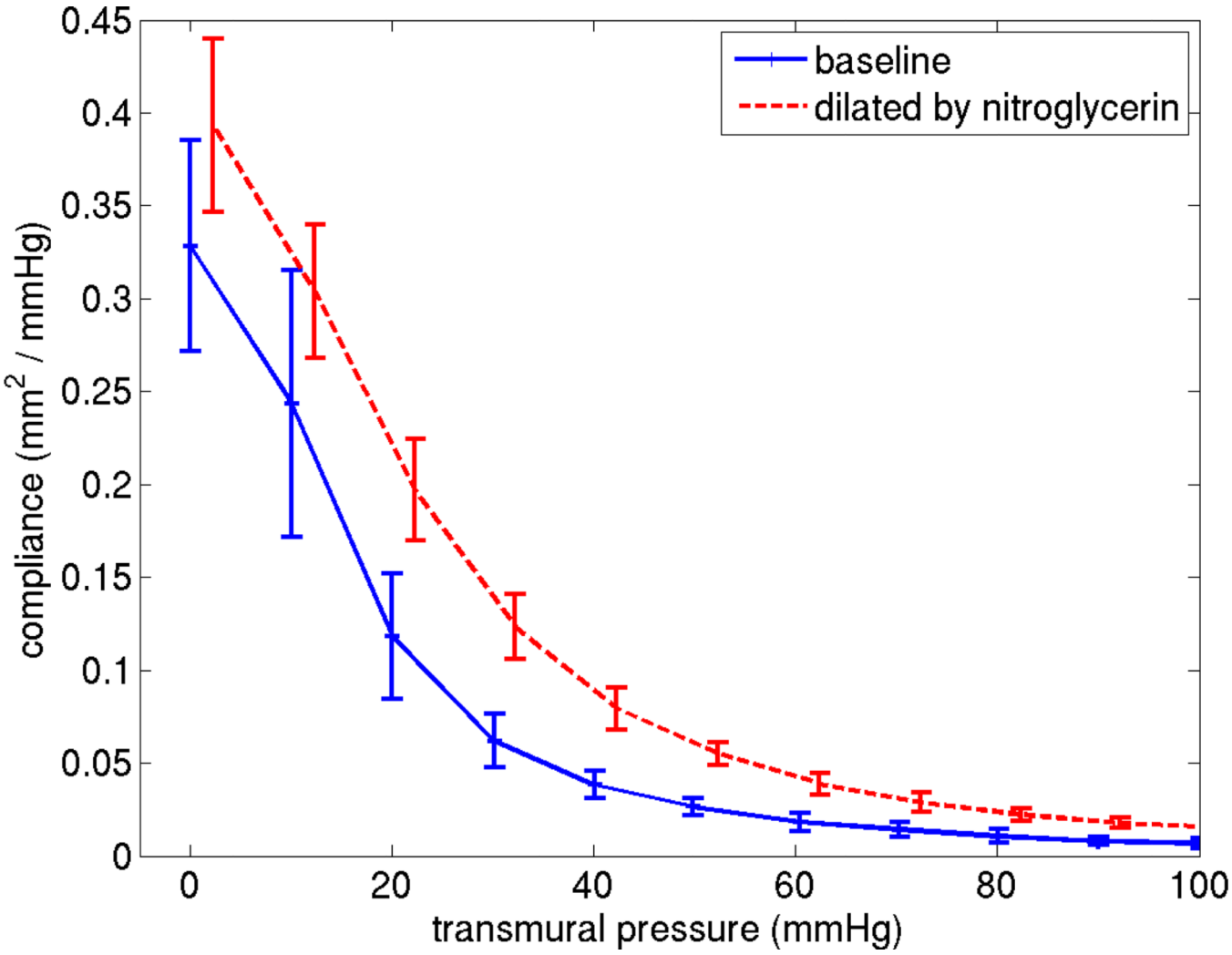

Figure 2.

Curves of compliance vs. transmural pressure in the brachial artery generated from data presented in [35]. According to this graph, the effect of relaxation of the arterial wall (here brought about by nitroglycerin) has maximal absolute influence on compliance when the transmural pressure is reduced to approximately 25 mmHg. This difference is much larger than that at full transmural pressure (right side of plot), and so is much easier to measure. This compliance difference between the baseline and dilated states also exhibits smaller variance ($p < 0.001$) at 25 mmHg of transmural pressure. Compliance values are shown ± the standard error of the mean. The error bars of the dilation series are interpolated to positions shifted rightward relative to the actual pressure values for visual clarity.

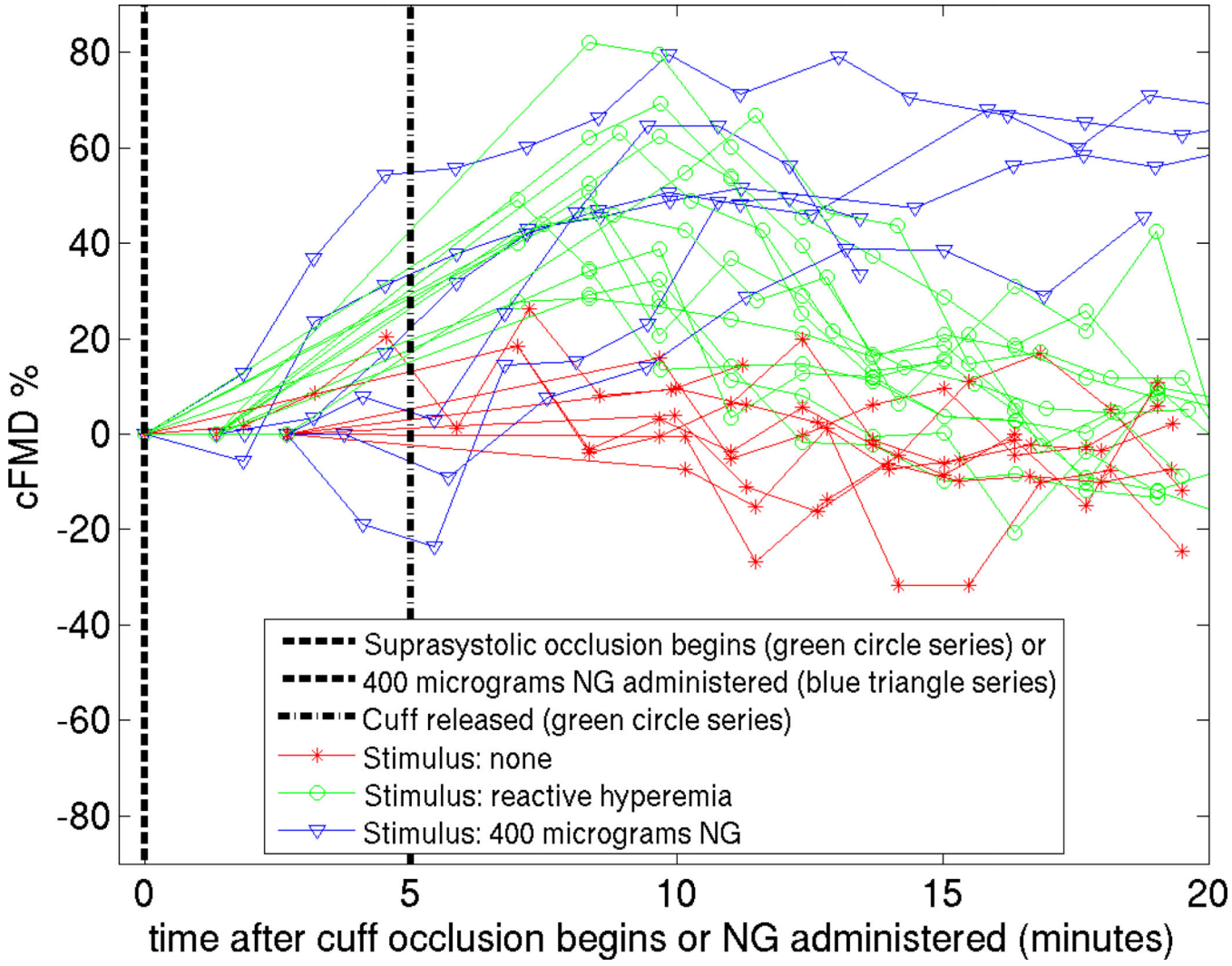

Figure 3.

Percentage change in cross-sectional area (2) observed relative to baseline for the preliminary studies listed in Table 2. Each sample point represents readings obtained during a T_m=30 s measurement period. It is clear that the method detects much larger changes in the cases where RH5 or NG is used as stimulus than when no stimulus is applied. The fact that there is a totally unambiguous distinction between the stimulus-present versus NS studies in all cases (time points in the range of $8 \leq t \leq 10$ minutes for RH5, and $10 \leq t \leq 20$ minutes for NG) is very encouraging.

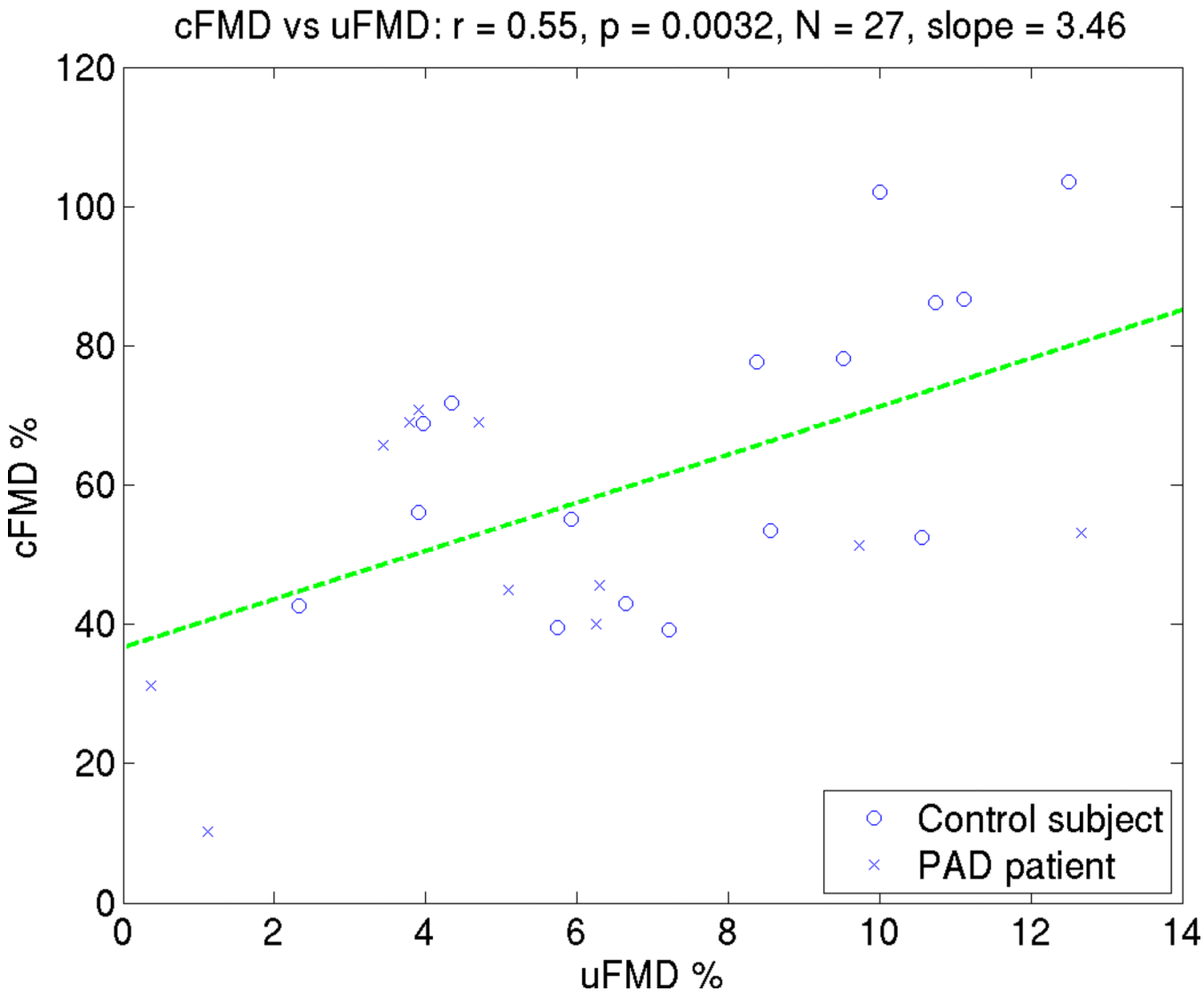

Figure 4.

Scatter plot of measurements of cFMD% vs. uFMD% for N=27 total subjects. We observe a correlation coefficient of r = 0.55, which is statistically significant with p = 0.003.

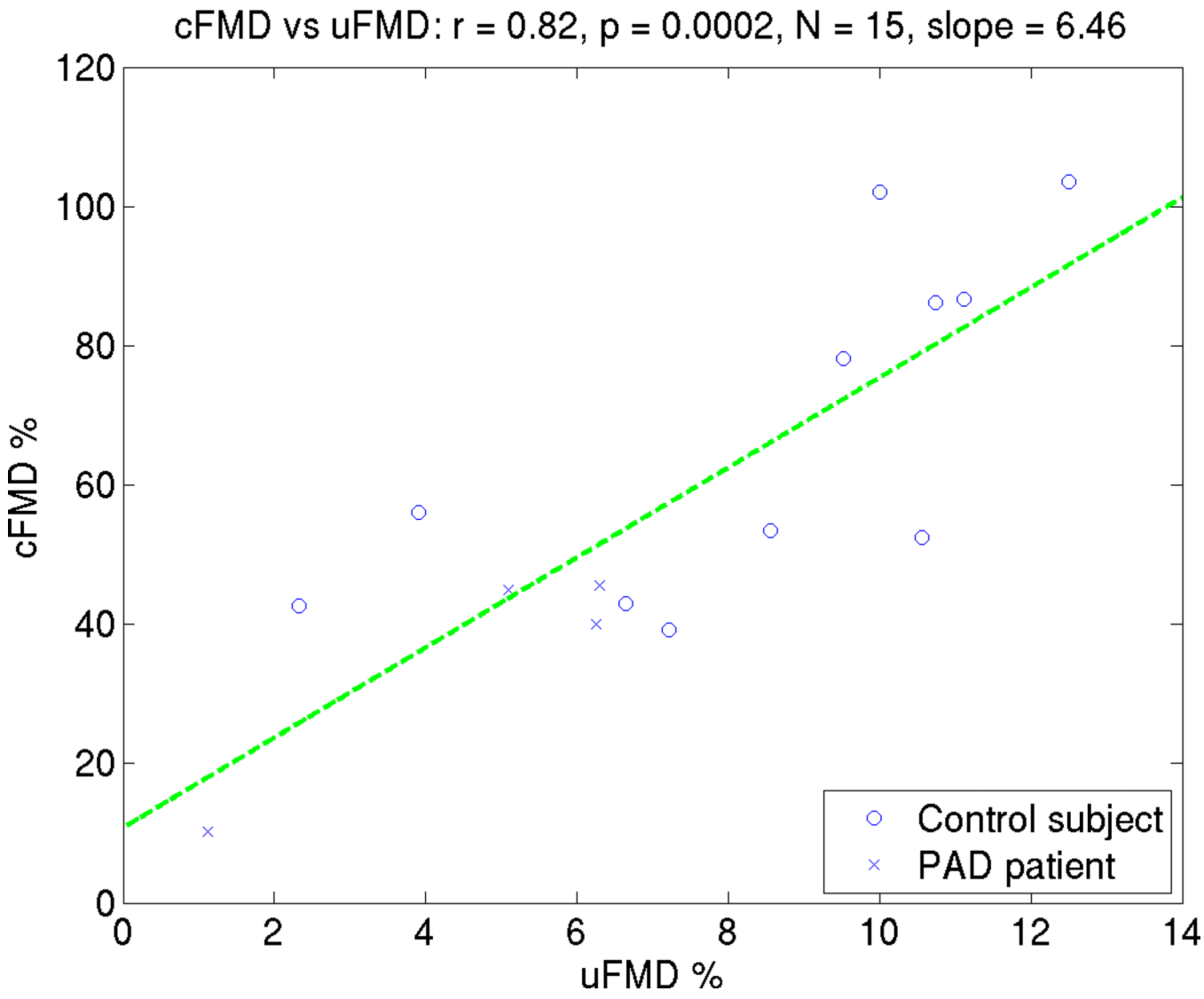

Figure 5.

Scatter plot of measurements of cFMD% vs. uFMD% for N=15 total subjects. We observe a correlation coefficient of r = 0.82, which is statistically significant with p = 0.0002. These subjects are the subset of those in Figure 4 that exhibited systolic blood pressures of less than or equal to 140 mmHg.

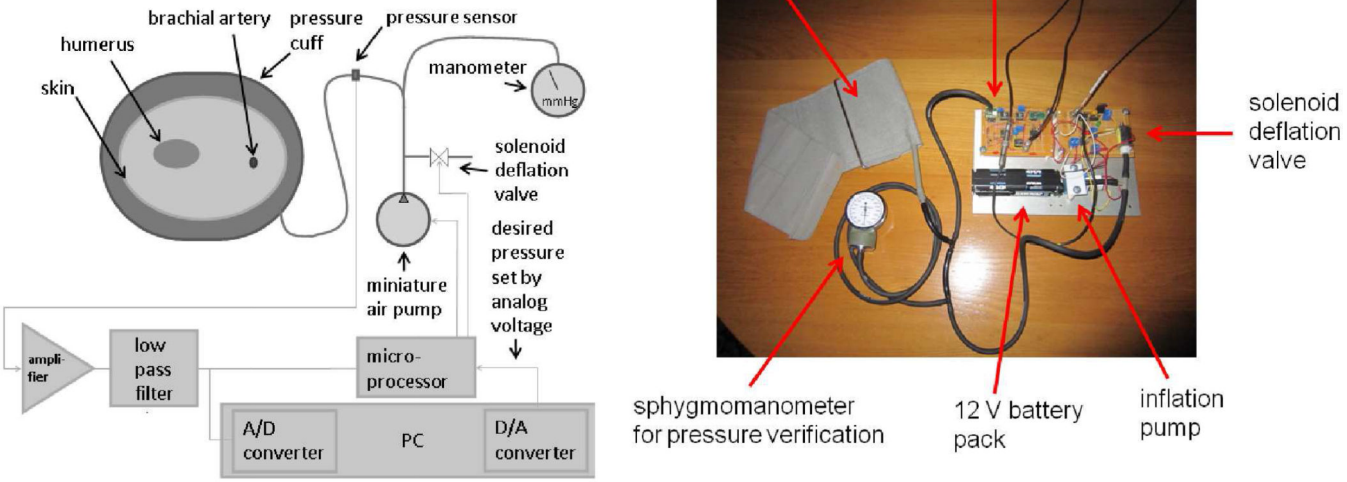

Figure 6.

Left: Schematic diagram of Prototype I. Analog signal conditioning and cuff pressure control reside in the instrument shown in the photograph (right). Data acquisition and processing are performed on an attached PC. **Right:** Photograph of Prototype I. The unit is connected to the data acquisition card of a PC (not shown) by three coaxial cables. Two carry the analog pressure output signals: the raw signal and a high-pass-filtered version that is used for display purposes only. The third conducts an analog input signal that determines the pressure set-point for the cuff. (MEMS: microelectromechanical system)

Figure 7.

Left: Photograph of an iHealth BP5 wireless blood pressure cuff. The cuff firmware is modified to allow users to execute the cFMD measurement protocol. **Right:** Measurement application running on an iPhone 5 that obtains the pressure waveforms from the cuff via Bluetooth.

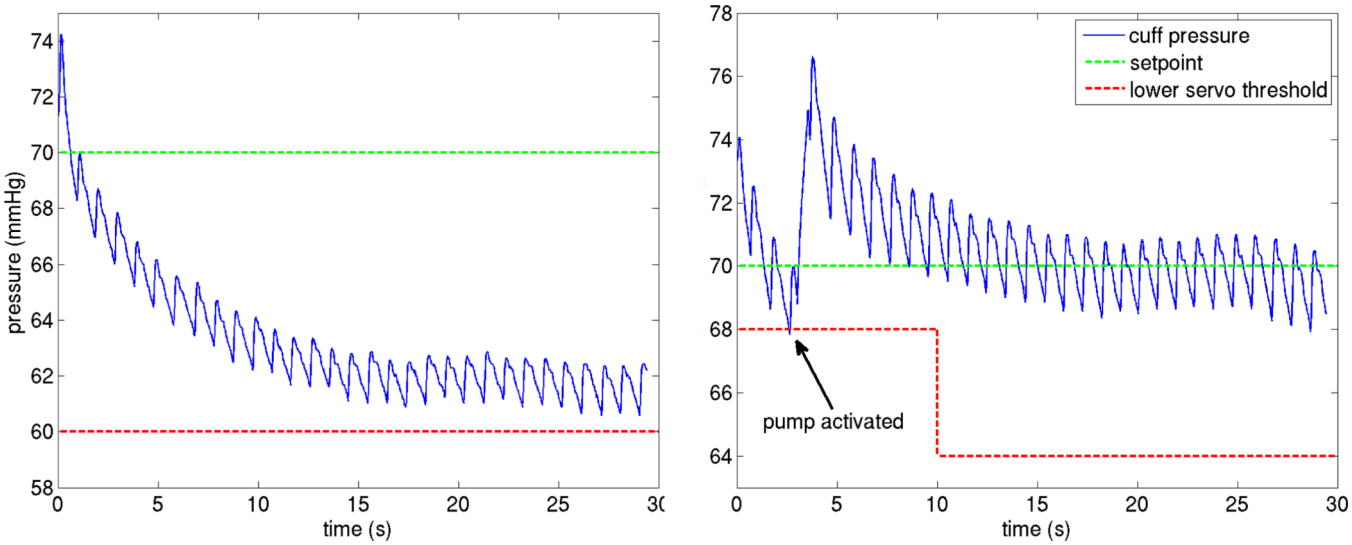

Figure 8.

Illustration of approaches to improve consistency of mean measurement pressure by addressing variations in pressure due to compression and conformation of the tissue under the cuff. The signal in the left panel is acquired with a large servo threshold pressure tolerances of $\Delta P_1 = \Delta P_2 = 10$ mmHg with respect to the setpoint of 70 mmHg. Subsidence of tissue under the cuff leads to a drop of over 7 mmHg below the set-point over the first 15 s. To yield the data in the right panel, the servo threshold is set to $\Delta P_1 = 2$ mmHg for the first $T_1 = 10$ s, and $\Delta P_2 = 4$ mmHg for the remaining $T_2 = 20$ s. These settings lead, in this case, to a stabilization of the signal close to the set-point during the first 10 s. While the relaxation of the pressure bounds during the last 20 s does not have an effect for this time series, it generally reduces signal disruption due to servo action during the later segment of the acquisition period.

Table 1

Table of abbreviations

AC	alternating current
BP	blood pressure
cD	vasodilation due to any stimulus, measured using cuff-based method
cFMD	flow-mediated vasodilation, measured using cuff-based method
CVD	cardiovascular disease
DC	direct current (mean signal value)
EDHF	endothelium-derived hyperpolarizing factor
EFMA	endothelial function in major arteries
FMD	flow-mediated vasodilation
NG	nitroglycerin
NO	nitric oxide
NOS	nitric oxide synthase
NS	no stimulus applied
PC	personal computer
RH	reactive hyperemia
RH5	reactive hyperemia after release of 5 minute occlusion
SEM	standard error of the mean
uFMD	FMD, measured using ultrasound imaging

Table 2

Subject characteristics for preliminary studies.

Subject number	Gender	Age	Framingham risk score	Number of studies NS/RH/NG
Subject 1	Male	38	1%	3/6/3
Subject 2	Female	38	<1%	4/3/3
Subject 3	Male	28	<1%	1/3/0

NS: no stimulus, RH: reactive hyperemia, NG: nitroglycerin

Table 3

Subject characteristics for cFMD/uFMD correlation study. Mean values are shown ± their standard deviations.

	All subjects included			Systolic hypertensives excluded		
	All	**Control**	**PAD**	**All**	**Control**	**PAD**
Number of subjects	27	16	11	16	11	5
# female	8	7	1	6	5	1
Age (years)	64.1±10.0	60.9±11.1	68.8±5.8	63.3±10.1	61.7±11.6	66.8±5.2
Mass (kg)	86.0±18.0	84.3±22.4	87.6±13.6	81.8±17.9	79.4±20.0	85.2±16.1
BMI (kg/m^2)	29.0±4.6	27.7±4.8	30.2±4.3	28.4±4.8	26.8±4.4	30.8±4.7
# diabetic	7	3	4	4	2	2
# tobacco ever	17	9	8	9	6	3
# tobacco current	6	3	3	3	2	1
Sysolic BP (mmHg)	144.8±23.1	135.5±13.4	158.3±28.0	130.6±7.5	129.5±8.1	133.2±5.9
Diastolic BP (mmHg)	87.3±9.8	86.6±10.2	88.4±9.5	82.2±4.9	82.5±4.8	81.6±5.6

Table 4

Statistical analysis of dilation response (cD_{max}%)

Stimulus	Mean ± SEM of maximum response over all datasets	cD_{max}%	p-value versus NS
RH	1.51 ± 0.052	51%	1.19×10^{-5}†
NG	1.70 ± 0.036	70%	6.25×10^{-6}†
NS	1.01 ± 0.068	1%	N/A

† statistically significant

SEM: standard error of the mean