A novel approach to the assessment of vascular endothelial function

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Abstract. Impaired endothelial function (EF) is associated with atherogenesis, and its quantitative assessment has prognostic value. Currently, methods based on assessing flow-mediated dilation (FMD) are technically difficult and expensive. We tested a novel way of assessing EF by measuring the time difference between pulses arriving at the middle fingers of each hand (f-fΔT), whilst FMD is induced in one arm. We compared f-fΔT with standard methods in healthy and diseased subjects. Our findings suggest that the proposed simple and inexpensive technique gives comparable results and has the potential to qualitatively assess EF in the clinical setting, although further work is required.

1. Introduction

Impaired endothelial function (EF) is an important early step in the atherosclerotic disease process and quantitative assessment of EF has prognostic value; hence, a readily available, non-invasive, method of assessment can provide a powerful screening tool for vascular disease [1].

The current gold-standard method of assessment uses duplex ultrasound to measure brachial artery flow-mediated dilation (FMD), which is dependent on the availability of nitric oxide released from endothelial cells. It follows that the greater the FMD, the better the EF. However, using duplex ultrasound is technically challenging, requires highly skilled operators, is expensive and therefore difficult to use as a readily available screening tool [2].

In an attempt to find an appropriate alternative, several novel biomechanical approaches have been explored, including measuring pulse wave velocity (PWV) to assess FMD and hence EF. PWV is the speed at which the pulse of blood propagates along vessels [3]. When a vessel dilates following vascular smooth muscle relaxation, its wall becomes acutely more compliant and the PWV decreases [4]. Therefore, measuring the PWV in the forearm (brachial-finger PWV, b-f PWV) whilst FMD is induced in the brachial artery (as a result of reactive hyperaemia), and comparing this to the b-f PWV when the arm was at rest, a quantitative measure of the brachial artery dilation can be derived. Studies have suggested that the assessment of FMD using this approach gives comparable results to that of the gold-standard duplex ultrasound method [5,6].

Yet another approach involves monitoring the pulses arriving at the middle finger (MF) of each hand, whilst the right arm has FMD induced in it and the left arm is at rest [7]. In this case, the time delay (f-f ΔT) between the arrival of the pulses at the right MF and the left MF will reflect a change in
the PWV and therefore dilation in the right brachial artery. Again, comparing this to the f-f ΔT of both arms at rest gives a quantitative measure of the dilation in the brachial artery. To our knowledge, use of this novel approach has not been reported yet.

The overall objective of this study is to validate the f-f ΔT method for the assessment of FMD. To do this we carried out five experiments, each of which, provided a partial validation. In Experiment-1 we tested the hypothesis that f-f ΔT will be greatest at the start of FMD and will decrease to resting levels as FMD diminishes with time; and that therefore, f-f ΔT will follow the same time course and magnitude as b-f PWV. In Experiment-2 we compared the assessment of FMD in healthy controls and thalassaemic adolescents using the b-f PWV approach and the f-f ΔT approach to see if these techniques could discriminate between healthy and diseased individuals. In Experiment-3 we tested the FMD response in healthy subjects and patients at risk of CVD using the f-f ΔT approach in order to see if it could discriminate between healthy subjects and subjects at risk of CVD. In Experiment-4 we assessed FMD in subjects with varying severity of coronary artery disease (CAD) using the established venous occlusion plethysmography (VOP) method and finally, in Experiment-5 we compared the assessment of FMD in normal, overweight and obese adolescents using the b-f PWV approach and the VOP approach. Therefore, in summary, our experiments were comparing the assessment of FMD using the three different techniques (f-f ΔT, b-f PWV and VOP) and also determining whether each technique could discriminate between different groups (normal and diseased subjects) on the basis of FMD assessment.

2. Method

2.1. Subjects

- Experiment-1: 10 healthy subjects with no CVD (mean [±SD] age 35±18.6 years) were recruited.
- Experiment-2: 30 controls (mean [±SD] age 14.4±1.2 years) and 29 patients with β-thalassaemia/Haemoglobin E (mean [±SD] age 13.8±1.5 years).
- Experiment-3: 28 healthy volunteers with low CVD risk (mean [±SD] age 21±1.29 years) and 12 patients with high CVD risk (mean [±SD] age 62±7.57 years) were recruited. “High CVD risk” implies having CVD or having CVD risk factors (diabetes mellitus, hypercholesterolaemia or smoking).
- Experiment-4: 33 CAD patients with risk factors (RF) ≤1, 46 CAD patients with RFs ≥2 and 71 CAD patients with RFs ≥3 were recruited. Mean [±SD] age for RFs ≤1 group was 63±1.4 years, for RFs ≥2 group it was 60±1.2 years and for RFs ≥3 group it was 62±1.0 years.
- Experiment-5: 54 male and 64 female adolescents were recruited and divided on the basis of BMI into 3 groups: 44 controls (mean [±SD] age 14.7±1.3 years) with a normal weight (mean BMI [±SD] 20.6±1.7); 35 overweight subjects (mean [±SD] age 14.5±1.4 years, mean BMI [±SD] 25.6±1.4) and 39 obese subjects (mean [±SD] age 14.5±1.6 years, BMI [±SD] 31.9±3.9).

2.2. Protocol

The standard method of inducing FMD was employed: a cuff placed around the upper arm is inflated to suprasystolic pressure to induce ischaemia for 5 minutes and, upon deflation of the cuff, hyperaemic flow induces endothelial-dependent dilation of the brachial artery [8].

For f-f ΔT measurements standard photoplethysmography (PPG) finger probes (Nelcor pulse oximeter probes) were used to monitor pulses at each middle finger (MF). For b-f PWV measurements a PPG finger probe and custom made flat PPG probes were used to monitor pulses at the MF and brachial artery. Alternatively, pulses at the brachial artery were monitored using 4 or 8MHz (as appropriate) ultrasound probes driven by a continuous wave Doppler flow velocimeter (Dopplex II, Huntleigh Healthcare, Cardiff, UK) in measurements for Experiments 2 and 5. The b-f distance was
measured with a flexible tape to the nearest 5mm for PWV calculation. One minute baseline f-f ΔT and b-f PWV were recorded before inflating the cuff so that post-occlusion values could later be compared to these baseline values to assess the magnitude and time course of any changes.

The PPG probes and Dopplex outputs were connected to a custom built arterial compliance monitor configured to accept inputs from a variety of probe types. The signals were captured at a rate of 1kHz, amplified and passed via a USB interface to a lap top computer running custom written software (PMD 3.0) which processed the signals and gave a real-time display of the pulses. The data were analysed offline using the PMD software.

Forearm blood flow (FBF) was measured by VOP according to previously described procedures [9,10] with some modifications. Briefly, a mercury-filled Silastic tube (D. E. Hokanson, Inc., Issaquah, WA, USA) was attached to the widest part of the forearm, and an occlusion cuff was placed just proximal to the elbow. Blood flow was measured following rapid inflation (<1 second) of the upper arm occlusion cuff to 40mmHg for 10 seconds to occlude venous outflow from the arm. The cuff was then allowed to deflate rapidly and remain deflated for 10 seconds. Each of these 20 second cycles was repeated 10 times. The FBF output signal was recorded and analysed with a BIOPAC data acquisition system (BIOPAC Systems, Goleta, CA, USA). Basal FBF values from the 10 inflation/deflation cycles were averaged. Reactive hyperaemia was induced by a brief period of ischemia achieved by inflating the upper arm cuff to a pressure of 200mmHg for 4 minutes. Following release of the high pressure occlusion, the FBF following reactive hyperaemia and its return to baseline was tracked by repeating the 20 second inflation/deflation cycles for a period of 3 minutes. The subjects were then allowed to rest for 10 min and the entire procedure was repeated.

2.3. Analysis

In experiments involving f-f ΔT or b-f PWV, two sets of pulses were obtained: pulses at right MF and pulses at left MF (f-f ΔT); and pulses at the brachial artery and pulses at the MF (b-f PWV). In both cases, the time delay between corresponding pulses in each set was determined by the foot-to-foot method (Figure 1). A mean f-f ΔT or b-f PWV for a time interval was calculated, and the absolute difference from baseline f-f ΔT or percentage change from b-f PWV was determined. In experiments involving VOP, the maximum increase in FBF during FMD (as a percentage change from baseline FBF) was calculated.

In Experiments 1 and 3, we used the time taken for the f-f ΔT or b-f PWV to return to baseline as the quantitative measure of brachial artery dilation. In Experiments 2, 4 and 5, the maximum change in f-f ΔT, b-f PWV or FBF was used for the quantitative measure of brachial artery dilation. In Experiment 4 we also used the area under the FBF-time curve as a quantitative measure of brachial artery dilation.

![Figure 1](image-url)

**Figure 1.** Images of pulses as seen on the PMD 3.0 software. The operator enters the height of each wave into the software and the software identifies the rising edge of the systolic wave (the ‘foot’) from the smoothed second differential of each signal. The foot-to-foot time (Δt) delay between the two signals is calculated for each heart beat.
3. Results

3.1. Experiment-1

![Figure 2](image_url)

**Figure 2.** The change in b-f PWV (left) and f-f ΔT (right) over the course of FMD. Error bars show standard error of the mean (SEM). Zero on each plot represents baseline values of b-f PWV and f-f ΔT. Between 0–30s there was a significant difference in b-f PWV from baseline and between 0–40s there was a significant difference in f-f ΔT from baseline f-f ΔT (p<0.05 on paired t-test).

3.2. Experiment-2

![Figure 3](image_url)

**Figure 3.** Maximum change from baseline in b-f PWV (left) and f-f ΔT (right) during FMD in controls and thalassaemia patients. Error bars show standard deviation (SD). * indicates significant difference (p<0.05 on t-test) between the groups.
3.3. Experiment 3

**Figure 4.** The change in f-f ΔT over the course of FMD in healthy volunteers and patients. Error bars show SEM. Zero on plot represents baseline f-f ΔT. Between 0–120s in healthy volunteers and 0–30s in patients there was a significant difference in f-f ΔT from baseline (p<0.05 on paired t-test).

**Figure 5.** The change in f-f ΔT over the course of FMD in healthy volunteers (left) and patients (right) after stratifying each group into sub-groups of those with mean arterial pressure (MAP) greater than and less than the group’s median MAP. Error bars show SEM. Zero on plot represents baseline f-f ΔT. There is significant difference (p<0.05 on t-test) between the healthy volunteer sub-groups at all times except 0s, 60s and 240s. There is significant difference (p<0.05 on t-test) between the patient sub-groups at all times except 0s, 60s and 180–600s.

3.4. Experiment 4

**Figure 6.** Maximum change from baseline in FBF during FMD in CAD patients with varying numbers of risk factors (left). Maximum percentage change from baseline in FBF and the area under the FBF-time curve during FMD in the same groups (right). Error bars show SEM. * indicates significant difference (p<0.05 on t-test) between the groups.
3.5. Experiment-5

Figure 7. Maximum change from baseline in FBF and b-f PWV during FMD for normal controls, overweight and obese subjects. Error bars show standard deviation (SD). * indicates significant difference (p<0.05) between the groups.

4. Discussion

As predicted, the change in f-f ΔT from the baseline f-f ΔT was greatest at the start of FMD and it decreased to baseline as FMD diminishes with time. This pattern was complemented by the b-f PWV curve which showed a drop in PWV before returning to baseline. Furthermore, the return to baseline in f-f ΔT and b-f PWV was at similar times (Figure 2). However, the b-f PWV data are highly variable when compared to the f-f ΔT data, which may reflect the relative ease of the f-f ΔT technique in obtaining stable signals using only PPG finger probes. Naka et al. showed that a significant drop in PWV is observed in the upper limb and lower limb during FMD [5], whilst Torrado et al. showed that a significant drop in carotid-radial PWV is observed during FMD [6].

Comparison of the b-f PWV and f-f ΔT methods during the assessment of FMD in thalassaemia patients also gave comparable results but the f-f ΔT method shows the responses in controls and thalassaemia patients are significantly different whilst this is not the case using the b-f PWV method (Figure 3). This may suggest that the f-f ΔT method is more sensitive than the b-f PWV method. However, since baseline f-f ΔT will tend to be near 0ms it is not numerically useful to calculate the percentage changes from baseline when using the f-f ΔT method, which is a limitation.

Using f-f ΔT to compare FMD in healthy volunteers (with low CVD risk) and patients (with high CVD risk) allowed us to test whether this novel approach is sensitive enough to detect the differences in EF, which we reasonably assumed existed, between the two groups. It took 120s for f-f ΔT (2.6±1.11ms [±SEM], p>0.05) to return to baseline in controls, whilst it took 30s for f-f ΔT (-6.2±2.61ms [±SEM], p>0.05) to return to baseline in patients (Figure 4). Therefore, using f-f ΔT, we were able to detect an attenuated FMD response in patients compared to the healthy volunteers. However it should be emphasised the mean age of the control group was very much less than that of the patients so much of the difference in response between the two groups could be due to age differences. However in Experiment 4, the groups were aged matched and attenuated response was seen in subjects with a greater disease risk, although measurements were confined to FBF. For both healthy volunteers and patients, sub-cohort analysis suggests that individuals with a lower MAP have a greater f-f ΔT change from baseline throughout FMD. This finding is statistically significant at most times throughout FMD, particularly in healthy volunteers and, to a lesser extent, in patients (Figure 5). Although, this suggest that the f-f ΔT approach is sensitive enough to spot such differences further work needs to be done to confirm this.

Using VOP to assess FMD in CAD patients with varying numbers of risk factors suggests that FBF can discriminate between high and low risk groups. There was a significant difference between the
RFs ≥2 vs. RFs ≥3 groups when comparing the FBF or the FBF-time curve area (but not when comparing the percentage change from baseline FBF). There was no significant difference between the RFs ≤1 vs. RFs ≥2 groups, and interestingly, none existed between RFs ≤1 vs. RFs ≥3 groups (the group we expected to have the largest difference) (Figure 6). When comparing FMD in normal, overweight and obese adolescents using the VOP and b-f PWV technique, significant differences between the groups in both FBF and b-f PWV were achieved. The maximum response (decrease from baseline) in b-f PWV during FMD is significantly greater in controls with a normal BMI than in overweight or obese adolescents. This is expected as obesity is a risk factor for CVD and therefore individuals would have impaired EF. The same is suggested by the maximum response in FBF using the VOP technique; normal controls have a significantly greater FBF than overweight and obese adolescents (Figure 7). However, one might expect obese individuals to have more impairment in their response than overweight individuals, however ANOVA followed by post hoc testing would be needed to investigate this further.

In this study we compared three techniques of assessing FMD in healthy and diseased subjects. The results suggest that the novel f-f ΔT method of assessing FMD, and hence EF, is simple and sensitive enough to be developed into a readily available screening tool. However, further work is required to elucidate some issues discussed here. Furthermore, the f-f ΔT changes observed during FMD still need to be compared to the FBF measured using VOP, and it also needs to be compared to direct measurements of diameter changes seen in FMD (using duplex ultrasound or MRI, the current gold-standard methods for assessing EF). Other simple approaches to assessing EF have been reported, including the measurement, during FMD, of the time delay between the ECG R wave and the arrival of the pulse wave at a peripheral site [11].

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