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Reproducibility and variability of digital thermal monitoring of vascular reactivity

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Summary

Background: Previous studies demonstrated that digital thermal monitoring (DTM) of vascular reactivity, a new test for vascular function assessment, is well correlated with Framingham Risk Score, coronary calcium score and CT angiography. This study evaluates the variability and reproducibility of DTM measurements. We hypothesized that DTM is reproducible, and its variability falls within the accepted range of clinical diagnostic tests.

Method: A fully automated DTM device (VENDYS, Endothelix Inc., Houston, TX, USA) was used for repeated measurement of vascular function in 18 healthy volunteers (age 35 ± 4 years, 74% men) after 24 h. All subjects underwent overnight fasting, and the test was preceded by 30-min rest in a supine position inside a dimmed room with temperature 22–24°C. The measurements were obtained during and after a 2-min supra systolic arm-cuff occlusion–induced reactive hyperaemia procedure. As a part of this study, the Doppler ultrasound hyperaemic, low-frequency, blood velocity of radial artery and a fingertip DTM of vascular function were compared simultaneously. Postcuff deflation temperature rebound and area under the curve, DTM indices of vascular function, were studied.

Results: Temperature rebound area under the curve correlated closely with Doppler hyperaemic, low-frequency, blood velocity ($r = 0{\cdot}97$, $P = 0{\cdot}0001$). Day-to-day intra-subject variability was 6·2% for baseline temperature, 8·7% for mean blood pressure and 11·4% for heart rate. The coefficient of repeatability of temperature rebound and area under the curve were 2·4% and 2·8%.

Conclusion: In a controlled environment, the repeatability of DTM is excellent. DTM can be used as a reproducible and operator-independent test for non-invasive measurement of vascular function.

Introduction

Traditional risk factor–based method of assessing cardiovascular health (Framingham Risk Score) fails to identify very-high-risk (vulnerable) individuals who have extensive atherosclerotic plaques in coronary and other arteries, thereby at risk for a near future adverse event (Naghavi, 2007). Other existing modalities for cardiovascular risk assessment beyond the traditional risk factors include imaging techniques, such as CT, MRI and ultrasound, which are either expensive or pose radiation therefore cannot be repeated often. Thus, non-invasive screening vascular function tests could be used to identify abnormal arterial function as an option for advanced risk assessment in appropriately selected persons, particularly in those with multiple risk factors who are judged to be at intermediate (or indeterminate) risk. An appropriate combination of structural and functional imaging can be more useful and predictive (Naghavi *et al.*, 2007).

Impaired vascular function, both endothelial mediated and endothelial independent, has been associated with atherosclerosis, related clinical manifestations, such as coronary heart disease and stroke, and cardiovascular events (Celermajer *et al.*, 1992; Schachinger *et al.*, 2000; Suwaidi *et al.*, 2000). Traditional non-invasive methods of measuring vascular function including high-resolution ultrasound imaging of brachial artery reactivity and venous occlusion forearm plethysmography during an arm-cuff occlusion–induced reactive hyperaemia have shown poor reproducibility and are difficult to perform in clinical practice (Fehling *et al.*, 1999; West *et al.*, 2004a; Peretz *et al.*, 2007).

Recently, peripheral arterial tonometry (PAT) technique has been introduced as an easier method for the measurement of vascular function. The PAT technique compares pulse amplitude at the fingertips before and after a 5-min arm-cuff-induced reactive hyperaemia (Kuvin *et al.*, 2001). Digital thermal monitoring (DTM) of vascular function has been introduced as new non-invasive assessment of vascular function (Nachiket *et al.*, 2003; Ahmadi *et al.*, 2009a). Similar to the PAT technique, DTM is performed during an arm-cuff occlusion–induced reactive hyperaemia. Both tests have modestly correlated with brachial artery reactivity (Nachiket *et al.*, 2003). An advantage of the DTM method is that it measures both cutaneous microvascular and vascular reactivities that result in increased blood flow to the fingers because of reactive hyperaemia. Studies have shown that cutaneous microvascular reactivity may be mediated via nitric oxide pathways, and cardiovascular pharmacologic and non-pharmacologic therapies may improve skin microvascular reactivity (Joannides *et al.*, 1995; Rossi *et al.*, 2007).

A number of studies have observed that reduced reactive hyperaemia measured by DTM was associated with increased risk of atherosclerotic cardiovascular disease and predicts the burden of atherosclerosis risk factors measured by Framingham Risk Score and subclinical coronary atherosclerosis measured by coronary calcium score and CT angiography (Ahmadi *et al.*, 2008, 2009b).

The utility of DTM in clinical and research studies is dependent upon the reproducibility of the measures obtained by DTM. This study evaluates the intra-subject variability of DTM.

Methods

Study participants

The study population consisted of 18 participants who underwent [age 35 ± 4 years, range: 25–44 years, 74% men, body mass index (BMI) 23 ± 2 kg m^{-2}] who underwent a DTM measurement study within 1 day. As a part of this study, the low-frequency, Doppler ultrasound hyperaemic blood velocity of radial artery and a fingertip DTM of vascular function during and after reactive hyperaemia procedure were compared simultaneously. All patients were free of known cardiovascular disease and were normotensive, normoglycaemic, normocholesterolaemic, non-smokers, without coronary artery disease, family history of premature CHD, infection or systemic inflammation. The study protocol and consent forms were approved by the Institutional Review Board of Los Angeles Biomedical Research Institute at Harbor UCLA Medical Center, Torrance, CA, USA.

Study protocols

All DTM measurements were performed in the morning in a quiet and dimmed room and at a controlled ambient temperature between 22 and 24°C. Female participants were examined during the follicular phase of their menstrual cycle. Studies were conducted after an overnight fast of at least 10 h (water was permitted) and abstinence from alcohol, caffeine, any vasoactive medications, exercise, high-fat foods and supplemental vitamin C. The measurements were obtained with the subjects supine and after 30 min of rest. Each patient's blood pressure in the control arm was recorded in a sitting position, 5 min before the DTM test (IntelliSense® Professional Digital Blood Pressure Monitor, HEM-907XL; Omron Inc, Chicago, IL, USA). DTM of both hands was obtained during 3-min stabilization, 2-min cuff inflation to 50 mmHg greater than systolic blood pressure and 5-min deflation using an automated, operator-independent protocol (VENDYS-5000; Endothelix Inc., Houston, TX, USA).

Thermal changes before, during and after arm-occlusive reactive hyperaemia test were traced continuously in the fingertip of both occluded and non-occluded arm and digitalized automatically using VENDYS software.

Data acquisition

The device consists of a computer-based thermometry system (0·006°C thermal resolution) with two fingertip RTD (resistance temperature detector) probes designed to minimize the skin–probe contact area and fingertip pressure. These fast-response probes were attached to the pulp of the index finger on both hands. This system includes a common automated sphygmomanometer cuff, an automated cuff-inflation pump and an automated release valve to permit the non-invasive measurement of arterial pressure and the control of occlusive hyperaemia. Dual-channel temperature data were simultaneously acquired at a 1-Hz sampling rate. To provide for the computation of simultaneous, bidirectional, mean flow velocity, an 8-MHz Doppler ultrasound transducer was placed over the radial artery of the occluded arm while quadrature Doppler signals were recorded at a sampling rate of 22 050 Hz. The Doppler probe had a wide beam to fully insonify the vessel and a flat profile to maintain a constant transducer–artery angle to stabilize the relationship between blood velocity and Doppler shift frequency minimizing motion artefacts (Li, 2004).

DTM analysis

Digital thermal monitoring assessments were operator independent. DTM thermal probes were attached to the pulp of the index finger, and thermal changes because of cuff occlusion were automatically recorded with a 1-Hz sampling rate using VENDYS software. Recording session times for each DTM test were automatically recorded. The definitions for postdeflation temperature rebound (TR) and area under the curve (TMP AUC) are shown in Fig. 1.

Doppler ultrasound measurement of hyperaemic blood velocity

To observe the relatively slow vascular, hyperaemic response, a low-frequency, Doppler ultrasound, mean flow velocity signal was computed, which eliminates the common, higher-fre-

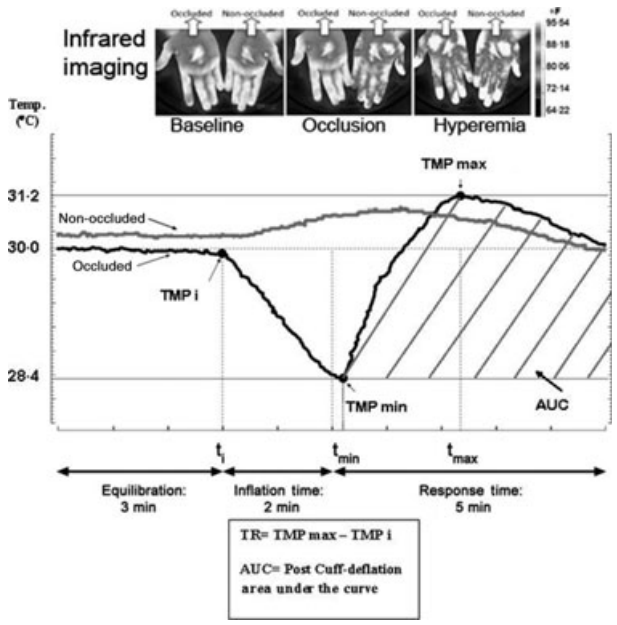

Figure 1 Skin temperature changes during cuff-reactive hyperaemia, as shown by infrared imaging and digital thermal monitoring (DTM).

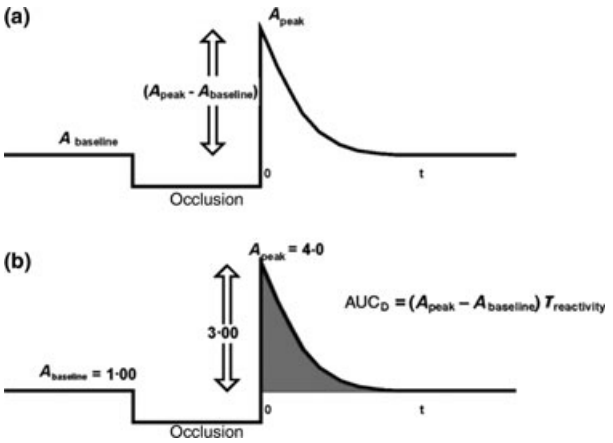

Figure 2 (a) A theoretical Doppler, low-frequency mean flow velocity signal during the measurement procedure with baseline amplitude, $A_{baseline}$, prior to cuff occlusion, zero flow during cuff occlusion, and hyperaemic response following cuff release showing peak amplitude, A_{peak}, and an exponential decay; (b) a low-frequency, Doppler flow signal normalized to baseline amplitude with the post cuff deflation area under the curve, AUC_D, (shaded red) equal to the product of the exponential amplitude ($A_{peak} - A_{baseline}$) and the reactivity time constant, $\tau_{reactivity}$.

quency, systolic and diastolic cardiac flow components. This desired low-frequency signal was obtained via digital signal processing methods by computing the bidirectional, mean Doppler shift frequency (Hz) across the vessel (75-Hz sampling rate) from the recorded Doppler quadrature signals; converting to a mean flow velocity signal (cm s^{-1}) via the common Doppler equation and knowledge of probe frequency and geometry; and applying a finite impulse response (FIR), linear-phase, lowpass filter with a cut-off frequency of 0·1 Hz. This low-frequency flow velocity signal (cm s^{-1}) may be further transformed to a flow signal (ml min^{-1}) via multiplication by the estimated cross-sectional area of the radial artery and conversion of appropriate time units. The area under the flow curve, expressed in units of fluid volume per time, becomes simply a fluid volume (ml) because a given area is the product of flow (ml min^{-1}) and time (min). Thus, the area under the Doppler flow curve immediately following the release of cuff occlusion, which is in excess of the baseline flow amplitude, represents an additional volume of blood introduced by reactive hyperaemia. When the flow curve, expressed in units of either Doppler shift (Hz), velocity (cm s^{-1}) or flow (ml min^{-1}), is normalized by the baseline flow amplitude, the normalized area under the Doppler flow curve, AUC_D, represents a normalized measure of this hyperaemic blood volume. For the theoretical flow curves of Fig. 2, this Doppler area under the curve, AUC_D (shaded in red), may be expressed as the product of the exponential amplitude, ($A_{peak} - A_{baseline}$), and the reactivity time constant, $\tau_{reactivity}$. Additionally, because blood is the primary heat transport mechanism within the body, the low-frequency, mean flow signal (ml min^{-1}) that indicates reactive hyperaemia also provides a dynamic measure of heat input into the finger and a basis for monitoring reactive hyperaemia via DTM sensors.

Generation of flow signals from DTM signals

Preliminary signal processing indicates that the heat transfer into the finger, proportional to finger blood flow, may be accurately represented by the low-frequency, mean flow signal after lowpass filtering. The characteristics of the lowpass filter are represented in the time domain by an exponential impulse response having a decay time constant equal to the DTM signal decay during cuff occlusion. Conversely, a normalized flow signal may be computed from a normalization of the DTM finger temperature deconvolved (inverse filtering) with the same exponentially decaying impulse response. Figure 3c shows the comparison of normalized flow waveforms obtained from Doppler sensors and DTM sensors. The preliminary similarity of these normalized signals supports the hypothesis that DTM and Doppler flow velocity signals are closely related during reactive hyperaemia procedures (Fig. 3c).

Statistical analysis

The descriptive data are presented as mean ± SD. Baseline temperature (TMP initial), TR and AUC were compared between repeated DTM measurements using t-test. Variability and repeatability of the test were measured and compared between repeated DTM measurements. Assessment of variability included the calculation of the coefficient of variation (CV) of the TR and AUC for repeated DTM measurements as well as day-to-day variability of the participants. Repeatability of DTM analysis for each cuff location was calculated using intra-class

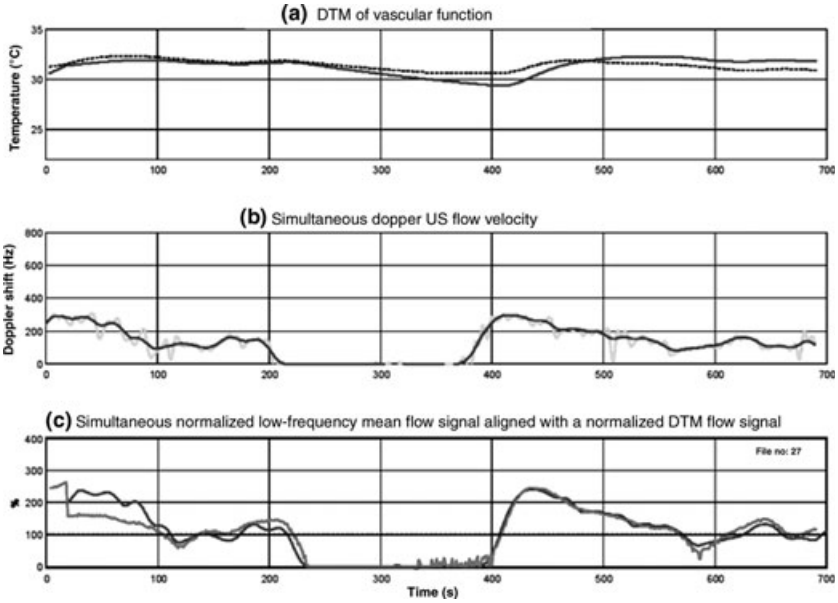

Figure 3 Vascular reactivity induced by reactive hyperaemia procedure: (a) Digital thermal monitoring (DTM) of vascular function in occluded (solid) and non-occluded (shaded) fingers; (b) simultaneous low-frequency mean flow velocity computed from Doppler signals; and (c) simultaneous, normalized low-frequency mean flow signal (blue) aligned with a normalized DTM flow signal (red) computed via a deconvolution of the DTM signal and an exponential impulse response generated with an estimate of the DTM decay time constant during cuff occlusion.

correlation coefficient (ICC) in a two-way mixed effects model and Bland & Altman, 1986 plots with calculation of the repeatability coefficient. SPSS 15.0 (Chicago, IL, USA, http://www.spss.com) statistical software was used.

Results

The TMP AUC correlated closely with Doppler ultrasound hyperaemic blood velocity, AUC_D (Figs 3 and 4). The mean baseline fingertip temperature was not significantly different in the 1-day repeat study (31·7 ± 2·7 versus 31·5 ± 2·8°C, P = 0·6). Additionally, the mean arterial blood pressure and heart rate were not different in day-to-day measurement (95·7 ± 5·4 versus 94·1 ± 6·1, P = 0·5 and 68·5 ± 4·5 versus 69·8 ± 3·1, P = 0·4, respectively).

The final fingertip temperature (300 s postcuff deflation) was equal to or lower than baseline temperature. The time of temperature minimum (TMP min) to temperature maximum (TMP max) was 150 ± 10 s. Mean TR and TMP AUC were similar after the repeat study (1·13 ± 0·22 versus 1·15 ± 0·21, P = 0·87, and 148 ± 23 versus 152 ± 24, P = 0·4, respectively).

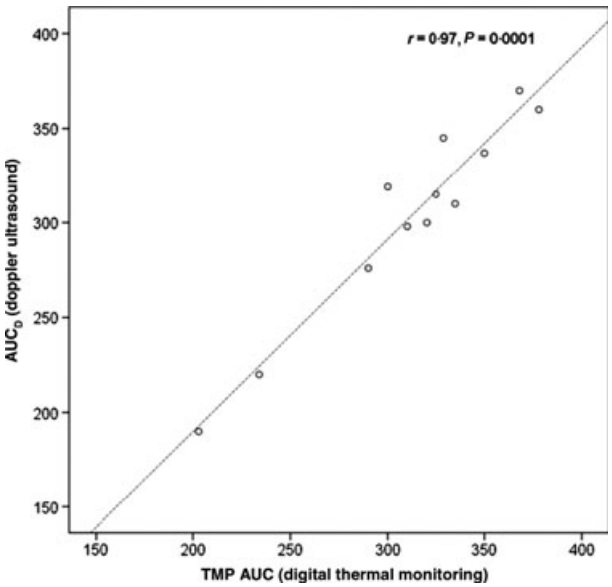

Figure 4 the correlation of AUC_D (Doppler ultrasound) and TMP AUC (digital thermal monitoring) (AUC = area under the curve, TMP = temperature).

Variability and repeatability

The day-to-day variability of mean arterial BP and heart rate was small (CV: 8·7% and 11·4%, respectively). Similarly, the day-to-day variability of TMP initial variation was small (CV = 7·1%). In addition, the repeated TR and TMP AUC were well correlated (Fig. 5), and day-to-day variability of repeated TR and TMP AUC was small (CV = 5·7 and 4·8%, respectively; Fig. 6). The repeatability of the test for TR and AUC was derived using Bland–Altman models that are shown in Table 1. The coefficient of repeatability (CR) of TR and TMP AUC was 2·4% and 2·8%, respectively. The ICC between TR of two repeated measurements was 0·82 (95%CI: 0·61–0·93, P = 0·0001); similar ICC for TMP AUC was also observed (0·83, 95% CI: 0·63–0·94, P = 0·0001).

Discussion

The main findings of this study are as follows: (i) measurements of baseline temperature are highly reproducible and reliable, with low coefficients of variation (7·1% for day-to-day variation); (ii) within-subject variability in DTM-based mea-

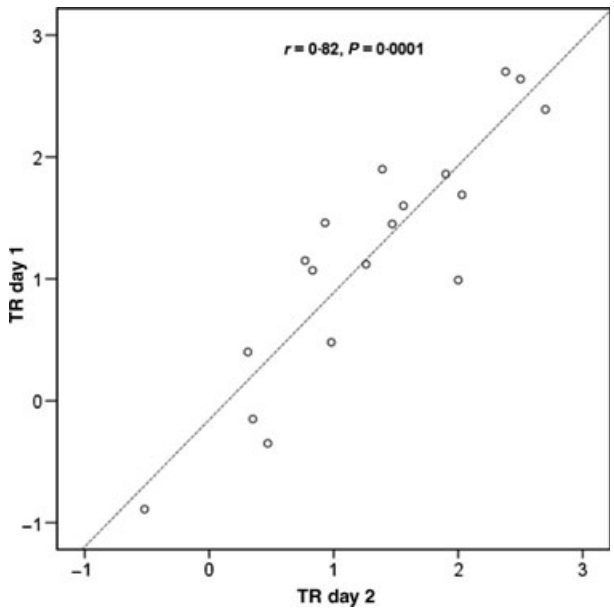

Figure 5 The correlation of repeated TR and TMP AUC (r = 0·83, P = 0·0001) (not shown in the figure) (TR = temperature rebound, TMP AUC = temperature area under the curve).

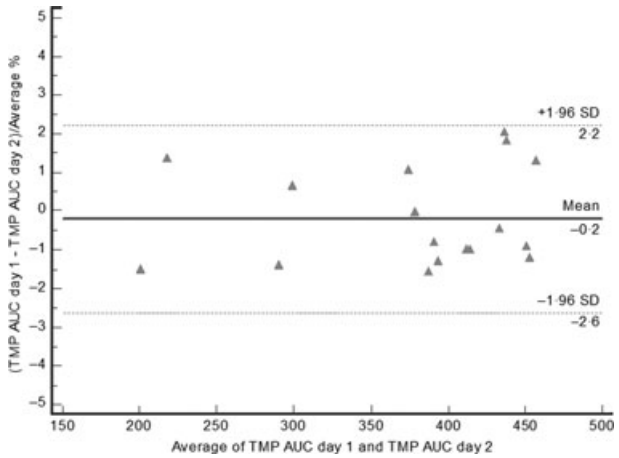

Figure 6 The variability of repeated digital thermal monitoring measurement for TMP AUC and temperature rebound ($y = -0{\cdot}031*X + 0{\cdot}057$) TMP AUC = temperature area under the curve.

surement of vascular reactivity (TR and TMP AUC) is low, suggesting similar responses to reactive hyperaemia stimulus; (iii) the low variation in DTM repeatability; and (iv) close correlation of the low-frequency blood flow and the DTM of vascular reactivity suggests DTM as an appropriate test to evaluate vascular reactivity in clinical and research settings.

Physiological estimates of the severity of vascular dysfunction can guide clinical decision-making to approach patients with coronary artery disease. Although the use of the intracoronary Doppler flow measurement and biplane angiography reliably determines coronary microvascular dysfunction, there is a need to develop tools that can assess vascular function non-invasively.

Table 1 Reproducibility and variability of digital thermal monitoring (DTM) of vascular function.

Variable	D	SD_D	CV (%)	CR (%)	ICC	*P* value
Heart rate	0·47	0·054	11·4	10·6	0·70	0·01
Mean arterial pressure	0·44	0·038	8·7	7·5	0·79	0·0005
TMP initial (°C)	0·51	0·036	7·1	7·1	0·81	0·0001
DTM indices of vascular reactivity						
TR (°C)	0·209	0·012	5·7	2·4	0·82	0·0001
TMP AUC	0·292	0·014	4·8	2·8	0·83	0·0001

D, mean absolute difference; SD_D, SD of mean differences; CV, coefficient of variability [$(SD_D/D) \times 100$]; CR, coefficient of repeatability [$(SD_D \times 1{\cdot}96) \times 100$)]; ICC, intra-class correlation coefficient.

The techniques employed to assess vascular function are as follows: ultrasound to quantify flow-mediated dilation (FMD), venous occlusion forearm plethysmography, reactive hyperaemia peripheral arterial tonometry (RH-PAT) and postocclusive reactive hyperaemia (PORH) test with laser Doppler flowmetry during reactive hyperaemia. The equipment, operator and physiological changes (e.g. the duration and type of stimulus, season, exercise, mental stress, food intake, menstrual cycle and cardiovascular risk factors; Deanfield *et al.*, 2007) contribute to the variability of measuring vascular function. Each of these modalities is plagued by problems of inter-test reproducibility. Inter-session variations for FMD responses (range 1–84%; West *et al.*, 2004b), venous occlusion forearm plethysmography (range 12–24%; Burggraaf *et al.*, 2000), RH-PAT (14·8–15·9%; Yvonne-Tee *et al.*, 2005) and PORH test with laser Doppler flowmetry (range 10·5–17%; Bircher *et al.*, 1994; Corretti *et al.*, 2002) limit their use as a 'therapeutic target' for an individual patient.

Flow-mediated dilation and venous occlusion forearm plethysmography are uniquely challenging, and the examination needs to be performed by well-trained, skilled individuals. Ergonomic factors are also important, with both the examiner and subject assuming comfortable positions, such that the transducer remains steady at the same anatomical position throughout (Bircher *et al.*, 1994; Corretti *et al.*, 2002).

Intra-/inter-observer, technical and interpretive variability, skew artefacts from cross-sectional imaging, temporal variability and brachial artery diameter limit accurate FMD assessments (Hijmering *et al.*, 2001; Alam *et al.*, 2005). Similarly, Intra-/inter-observer and technical and interpretive variability limit accurate measurement of vascular function with venous occlusion plethysmography (Donald *et al.*, 2008).

The variation of vascular function assessment with PORH test with laser Doppler flowmetry is substantial. Spatial and temporal variability and technical and interpretive variability decreased accuracy of PORH in vascular function assessment (Binggeli *et al.*, 2003).

Without restraint, recording of arterial tone is quite difficult because of motion artefact (hand or finger motion can appear in recording of arterial tone). Additionally, a change in sensor position or acceleration can result other type of artefact. The

artefacts are often comparable in magnitude and frequency to the pulse tone rendering, and simple filtering methods are useless. Above-mentioned artefacts and lack of automated compressor for controlled occlusion hyperaemia limit accurate vascular function assessment with RH-PAT (Agner & Serup, 1990).

Previous studies demonstrated that combined endothelial nitric oxide synthase (eNOS) and cyclooxygenase (COX) inhibition attenuated the acetylcholine (Ach)-induced, endothelium-dependent, cutaneous vasodilatation. In addition, the cutaneous vasorelaxant response to the endothelium-independent vasodilator, sodium nitroprusside (SNP), was unaffected. They suggested endothelium-dependent mechanisms for cutaneous vascular reactivity (Katz & Krum, 2001).

On the other hand, recent studies showed that simultaneous blockade of eNOS and COX has no effect on baseline skin blood flow and a considerable portion of the Ach-induced vasodilatation was preserved. These findings suggest that a non-nitric oxide, non-prostanoid-dependent pathway, potentially attributable to endothelium-derived hyperpolarizing factor (EDHF), plays an important role in the regulation of vascular tone in the microcirculation of human skin (Ludmer *et al.*, 1986).

The strong correlation between fingertip DTM of vascular reactivity and hyperaemic blood velocity as well as easy access to the skin microcirculation suggests that this simple and non-invasive modality can be reliably used to detect early vascular dysfunction without clinical manifestations and also monitor potential changes in vascular function in response to therapy or disease progression. DTM is a potentially useful non-invasive addition to current techniques to assess the underlying vascular dysfunction in a reliable manner because it uses standardized, operator-independent, automated measurements that can minimize equipment- and operator-related influences.

Conclusion

Evaluation of vascular function of the coronary arteries focuses on the circulation of clinical relevance (Schachinger *et al.*, 2000) and is a predictor of cardiovascular events (Celermajer *et al.*, 1992; Suwaidi *et al.*, 2000). Because vascular dysfunction is not confined to the coronary arteries, non-invasive techniques to assess peripheral vascular function have been developed and have been used in clinical studies to evaluate the responses to a particular therapy (Celermajer *et al.*, 1992; Schachinger *et al.*, 2000; Suwaidi *et al.*, 2000). DTM of vascular reactivity, with its low inter-session variability, could serve to stratify cardiovascular risk and monitor the vascular response to therapies.

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