Clin Physiol Funct Imaging (2011) **31,** pp66–72 doi: 10.1111/j.1475-097X.2010.00981.x

Use of temperature alterations to characterize vascular reactivity

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Summary

Monitoring alterations in fingertip temperature during ischaemia and the subsequent hyperaemia provides a novel way of studying microvascular reactivity. The relations between parameters characterizing blood perfusion and the thermal response of fingertips were studied using experimental and theoretical approaches. During the experimental protocol, two brachial artery occlusion tests were conducted in 12 healthy volunteers, and fingertip temperature, heat flux and skin perfusion using laser Doppler flowmetry (LDF) were measured. The temperature curves provide a smooth and robust response that is able to capture occlusion and reperfusion. The temperature fall during occlusion as well as the maximum temperature recorded depended linearly on the initial temperature. The magnitude of the LDF signal was associated with local tissue temperature and followed an exponential response. Heat flux measurements demonstrated rapid changes and followed variations in blood perfusion closely. The time points at which the heat flux reached its maximum corresponded to the time at which the fingertip temperature curves showed an inflection point after cuff release. The time required for the fingertip temperature to arrive at the maximum temperature was greater than the time to peak for the heat flux signal, which was greater than the LDF signal to reach a maximum. The time lag between these signals was a function of the finger size and finger temperature at the moment reperfusion restarted. Our present results indicate that finger temperature, heat flux and perfusion display varying rates of recovery following ischaemic stimuli and that differential responses are associated with the initial finger temperature.

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Accepted for publication

Received 29 June 2010;
accepted 30 August 2010

Key words

fingertip temperature; heat flux; hyperaemia; microvascular reactivity

Introduction

Cardiovascular disease (CVD) is responsible for more than a third of all deaths in the United States (Rosamond *et al.*, 2007). These statistics drive the need for developing vascular function tests that help identify asymptomatic individuals and monitor patients' responses to therapy. Vascular function assessments rely on measuring the magnitude of the hyperaemic flow following ischaemia, which is commonly produced by occluding a major artery such as the brachial artery. When vascular function is compromised, the dilatation following ischaemia (hyperaemia) is markedly reduced. This reduction is attributed to endothelial dysfunction, a globalized systemic disease process consisting of attenuated endothelium-dependent vasodilatation, augmented vasoconstriction and microvessel structural remodelling (Widlansky *et al.*, 2003).

In recent years, interest in monitoring functional changes in the microvasculature has increased substantially (Hartley & Tanaka, 2010) because of the growing number of reports suggesting that endothelial dysfunction or alterations in vessel tone manifest first in the microvasculature (Lavie *et al.*, 2000; Bonetti *et al.*, 2004). The main advantage of monitoring microvascular function is that a vascular technician is not required, and the analysis can be performed in the non-research environment. Common technique to assess microvascular function during reactivity tests is through the use of laser Doppler flowmetry (LDF) (Svensson & Jonsson, 1988; Kurvers *et al.*, 1995). Although LDF has been widely used in the literature (Svensson & Jonsson, 1988; Kurvers *et al.*, 1995; Kruger *et al.*, 2006), it provides only relative perfusion measurements, the equipment has inherently high cost, the measurement is dependent on the optical properties of the tissue, which can vary greatly with anatomical location and among subjects (Cracowski *et al.*, 2006). Other factors that affect the magnitude of a hyperaemic response in an individual depend on initial skin temperature, level of ischaemia produced

by either occlusion time or occlusion pressure and the presence of pain or discomfort that are associated with vasoconstriction (Kidd & Mc, 1958; Ozisik & Orlande, 2000). These factors are known to affect the reproducibility of the hyperaemic response (Savage & Brengelmann, 1994; Cracowski *et al.*, 2006). To reduce these effects, protocols typically involve heating in the probe location to a temperature of maximum dilatation (42°C) (Savage & Brengelmann, 1994), and rigorous control of occlusion time, pressure and posture. Requiring such strict experimental conditions makes this technique unsuitable for implementing in clinical practice.

A new device called the digital thermal monitoring (DTM) technique was developed to assess vascular reactivity in the microvasculature (Ahmadi *et al.*, 2008, 2009; Dhindsa *et al.*, 2008). This technique relies on the premise that the hyperaemic response following arterial occlusion can be characterized by recording and analysing temperature alterations at the fingertips. Reductions in the hyperaemic response, a hallmark feature of impaired vascular function, are observed as reductions in temperature rebound (TR) during thermal monitoring. Recent studies have demonstrated the ability of the DTM test to discriminate individuals with established CVD or high risk of future CVD in normal and low-risk individuals (Ahmadi *et al.*, 2008, 2009). The DTM technique is easy to set up and administer, does not require an expensive machine or a vascular technician and can be used to assess large populations in a clinical setting. A drawback of the DTM technique is that fingertip temperature depends not only on local skin perfusion but also on ambient temperature as well as the thermoregulatory capabilities of the subject.

Accordingly, the present study determined the associations between the thermal response of the fingertips (tissue temperature, heat flux and their temporal alterations) during reactivity tests and parameters commonly used to describe postocclusive hyperaemia in LDF studies, including baseline perfusion, magnitude of the hyperaemic response and time to peak. LDF is chosen as the standard to characterize microvascular function because 80% of blood in fingers is stored in the skin (Burch, 1954) and strongly affects skin temperature variation. We reasoned that the correlations found between parameters characterizing the thermal response and those characterizing the hyperaemic response recorded using LDF would strengthen interest in thermal monitoring for the assessment of vascular function. To more comprehensively address this issue, we used both experimental analysis and a mathematical model of heat transfer to analyse the contribution of different factors to the recorded skin temperature and to show its correlation to the characteristics of the hyperaemic response.

Methods

A total of 12 apparently healthy subjects (three women and nine men) were studied. The subjects had mean age and body mass of 28 ± 5 years and 68 ± 11 kg. All subjects were non-smokers, non-obese and free of overt cardiovascular or other chronic diseases as assessed by medical history. None of the subjects were taking cardiovascular-acting medications. The Human Research Committee at the University of Texas at Austin reviewed and approved all procedures, and written consents were obtained from all subjects.

Subjects refrained from all caffeinated drinks and food consumption for at least 4 h prior to the test. After the protocol was explained, subjects went through a stabilization period lasting between 25 and 45 min during which all sensors were placed. Subjects rested in a supine position in a quiet, dimly lit, temperature-controlled (22–24°C) room, with the right arm extended and placed in an arm support device commonly used in flow-mediated dilatation studies (Dhindsa *et al.*, 2008) that maintains the palm of the hand facing up and reduces unwanted mobility. Foam blocks were placed below the hand at wrist level to avoid contact with the metallic surface of the device and to increase comfort of subjects. The protocol included two vascular reactivity tests; each test consisted of 5–7 min of baseline, upper arm arterial occlusion for 5 min using a pneumatic cuff inflated to 100 mmHg over systolic blood pressure of the subject, and recovery for 5–10 min after occlusion. The two arterial occlusions were separated by 25–45 min of rest, which allowed subjects to fully recover and for values to return to baseline.

During each reactivity test, three signals were recorded on the pulp of different fingers: skin temperature, skin heat flux and skin perfusion. Fingertip temperature was measured on the index finger in both the occluded and non-occluded (or contralateral) arms (VENDYS; Endothelix, Houston, TX, USA). Skin temperature was recorded using ANSI type 'T' thermocouples (Copper/Constantan) that measure temperature at ±0·3°C over the range of 20–36°C. The system has a sensitivity to detect temperature changes of ±0·01°C. The thermocouple junction has very low mass to minimize effects of thermal transfer to and from the fingers and is laminated with kapton polyimide (thickness of 0·05 mm) on the measurement surface and a fibreglass-embedded Teflon on the opposite side. Sensors were kept in place at the fingertips by a polyethylene expandable mesh sleeves that encapsulated the fingertips and minimized exposure to the surrounding environment. Junctions were pressed against the skin surface using a plate of kapton polyimide, positioned inside of the polyethylene expandable mesh sleeves.

In the DTM test (Dhindsa *et al.*, 2008), the parameters characterizing the temperature signal correspond to the initial or starting fingertip temperature at the moment of cuff inflation (T_{start}), the lowest temperature (nadir) observed after cuff inflation (T_{min}), the highest temperature observed after cuff deflation (T_{max}), the temperature fall ($TF = T_{start} - T_{min}$), the rebound temperature after occlusion is released ($TR = T_{max} - T_{start}$) and the temperature increase ($TI = T_{max} - T_{min}$) were measured on both occluded and non-occluded sides.

Heat flux was monitored at the middle finger of the occluded arm only using heat flux sensors (FMR-200T; Wuntronic Inc., Munich, Germany) according to previous studies indicating a

high correlation between heat flux at the skin surface, skin temperature and blood perfusion (Scott *et al.*, 1998; Ricketts *et al.*, 2008). A flat plate transducer designed to measure heat flow was attached to the skin surface. The sensor provides a measurement of millivolts (mV) directly proportional to the heat flux flowing through it. Heat flux was recorded at the same sampling frequency as the fingertip temperature. Skin blood perfusion was measured at the fingertip of the ring finger in the occluded arm using the MoorLab LDF system (Moor Instruments, Devon, UK), where the Doppler shift of the scattered laser light is interpreted as a measure of red blood cell flux or perfusion. The LDF signal was measured and expressed in mV.

Data analysis

Characteristic temperature, heat flux and perfusion signals during a typical test are shown in Fig. 1. In these plots, three distinct stages are identified (separated by dashed vertical lines): baseline lasting 5–7 min, arterial occlusion for 5 min and postocclusion characterized by marked hyperaemia.

Fingertip heat flux (Q) was reduced upon arterial occlusion as the finger became progressively colder, and the difference in temperature between the surrounding air and the tissue surface was gradually reduced. During early reperfusion, the heat flux increased until it reached a maximum, the magnitude of which depended on the initial fingertip temperature. Positive values of Q indicate that heat is transferred from the skin surface to the environment (heat is lost) because the fingertip temperature is higher than that of the surrounding air (Scott *et al.*, 1998; Ricketts *et al.*, 2008). The heat flux signal is characterized by the time required to attain the peak ($t_{p,Q}$) and the maximum heat flux value (Q_{max}). The heat flux signal recorded is proportional to the time derivative of the fingertip temperature as observed from the mathematical model of Eq. (1).

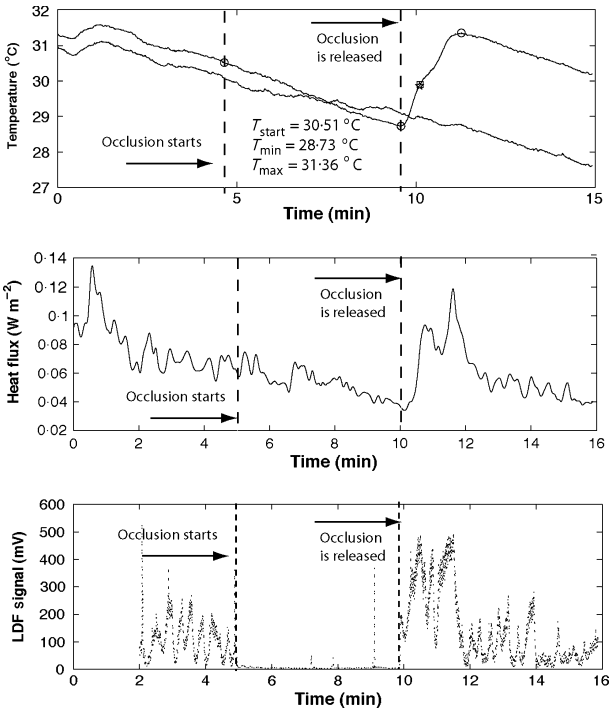

Figure 1 Representative temperature, heat flux and laser Doppler flowmetry signals during a test. Horizontal dashed lines mark the occlusion time (5 min).

The LDF signal acquired during the reactivity tests is characterized by basal perfusion (LDF_o), maximum perfusion (LDF_{max}) and time to peak ($t_{p,LDF}$). These parameters are obtained after filtering the raw LDF signal using the empirical mode decomposition (EMD) method (Assous *et al.*, 2005) to the LDF signal. Studies of hyperaemic LDF signals indicate that accurate LDF parameters can be determined from the fifth intrinsic mode function component (Assous *et al.*, 2005).

Theoretical analysis of fingertip temperature

Blood flow in the hands and fingers is affected by local skin temperature, systemic temperature and ambient temperature (Chato, 1980; Ducharme & Tikuisis, 1994; Shitzer *et al.*, 1996). To analyse and estimate the fingertip temperature changes experienced in response to different perfusion behaviours, a simplified mathematical model of heat transfer previously developed to analyse fingertip temperature changes during DTM was used (Ley *et al.*, 2008; Ley & Deshpande, 2009). This model considers two main contributions: the heat delivered to the finger by the circulation (Ducharme & Tikuisis, 1994) and the environmental heat loss (Shitzer *et al.*, 1996). Details about the approximations for this model, as well as its ability to reproduce the fingertip temperature during the DTM test, are discussed elsewhere (Ley & Deshpande, 2009). The governing equation takes the form:

$$\rho C_p V \frac{dT}{dt} = \rho_b C_{pb} \omega_b(t,T)(T_A - T) - h_{air} A (T - T_{air}) \qquad (1)$$

where ρ is the tissue density, C_p is the tissue specific heat, ρ_b and C_{pb} represent the density and specific heat of blood, and $\omega_b(t,T)$ indicates the volumetric blood perfusion and its temporal variation during the reactivity test (vascular occlusion and hyperaemic reperfusion) (Ley *et al.*, 2008; Ley & Deshpande, 2009). The coefficient h_{air} represents the heat transfer coefficient at the skin surface, h_{air} is influenced by geometric parameters such as finger size and length, surface area and volume, as well as posture and skin temperature (Kidd & Mc, 1958). T_{air} represents the temperature of the surrounding air, V represents the fingertip volume and A is the fingertip surface area. The temperature T_A is the temperature of the arterial blood entering the tissue. This temperature can be approximated to be constant and equal to core or body temperature (Pennes, 1948; Chato, 1980). The right-hand side of Eq. (1) corresponds to the rate of heat flow through the skin surface, which is proportional to the heat flux and can be directly recorded at the skin surface (Scott *et al.*, 1998).

To remove the parameters from the analysis (e.g. V and A), the Eq. (1) was rewritten as follows:

$$\frac{dT}{dt} = W(t,T)(T_A - T) - H(T - T_{air}) \quad (2)$$

where $H = h_{air}A/\rho C_p V$ and $W(t,T) = \rho_b C_{pb}\omega_b(t,T)/\rho C_p V$ represent coefficients characterizing the environmental and the perfusion contribution during a DTM test, respectively. Given the dependence of h_{air} with the skin temperature and anthropometric factors (Kidd & Mc, 1958), the coefficient H will vary among subjects. The temporal form for the perfusion function $W(t,T)$ is proportional to the LDF signal recorded during vascular reactivity studies (Svensson & Jonsson, 1988; Tee *et al.*, 2004), and during postocclusion (i.e. $t > t_s$, with t_s representing the time at which the arterial occlusion was started), it is described in terms of parameters such as the baseline perfusion (W_o), the maximum perfusion ($W_{max} = \beta W_o$), the time to peak (t_p) and the time necessary to return to baseline (τ_h) as described by the following equation:

$$W^{post-occ}(t) = \begin{cases} W_{max}\sin^2(\pi t/2t_p), & \text{for } t_{occ} < t \le (t_{occ} + t_p), \\ (W_{max} - W_o)\exp(-(t - t_p)/\tau_h) + W_o, & \text{for } t > (t_{occ} + t_p). \end{cases}$$

Substituting this equation for the perfusion into the model (Eq. 2), it is observed that the maximum of the time derivative of the temperature signal (dT/dt) occurs when the perfusion signal reaches a maximum $t = t_p$ and that point is associated with an inflection point in the temperature signal where both the time derivative of the temperature (dT/dt) and the temperature history [$T(t)$] are calculated using the model of Eq. (2) and the time function for W.

We have previously shown that the model describing the fingertip temperature history reproduces the observations during the DTM test (Ley *et al.*, 2008). In the present study, Eq. (2) and the recorded temperature were used to estimate the environmental contribution to fingertip temperature and to calculate the blood perfusion function $W(t,T)$. The environmental contribution to the fingertip temperature (H) is calculated using the analytical solution of Eq. (2) during occlusion [$W(t,T) = 0$], which provides the following equation for the temperature:

$$T(t) - T_{air} = \exp[-H(t - t_s)] \quad (3)$$

In this equation, T_{air} is the ambient or air temperature and t_s is the time at which occlusion was started. Equation (3) is valid in the range $t_s \le t \le t_{min}$ and can be used to correlate the starting T_{start} and the minimum temperature (T_{min}), as follows:

$$T_{min} = T_{start}\exp[-H(t_{occ})] + T_{air}(1 - \exp[-H(t_{occ})]) \quad (4)$$

where $t_{occ} = t_{min} - t_s$ indicates the duration of the occlusion period. Equations (3) and (4) together with the recorded fingertip temperature during occlusion can be used to estimate the environmental contribution to the fingertip temperature (H). In addition, Eq. (2) can be used to calculate the perfusion function (W) using the time derivative of the temperature signal and the calculated H.

Statistical analyses

Correlational and regressional analyses were used to determine the relations. Significance was set *a priori* at $P<0{\cdot}05$. All data were expressed as mean ± SEM.

Results

As indicated by Eq. (4), the slope of the plot T_{min} versus T_{start} (Fig. 2) was associated with the average environmental effect for all subjects and tests ($\bar{H} = 0{\cdot}0011 \pm 0{\cdot}0005\ s^{-1}$). To estimate the environmental effect during Test I (H_I) and Test II (H_{II}) for every subject, the recorded temperature during the occlusion period and Eq. (3) were analysed. A linear least squares procedure was applied to the plot $\ln[T(t) - T_{air}]$ versus $(t - t_s)$ obtained for each test to estimate the slope of the temperature line observed during occlusion. The subject-dependent H coefficient and its variation between Test I (H_I) and Test II (H_{II}) were associated with the starting temperature as well as the starting temperature difference between experiments (as the variation between H_I and H_{II} increased). Analysis of the subject-dependent H coefficient and its variations among the tests provides information about the effect of initial temperature and the repeatability of the thermal response.

The present protocol allowed us to test responses in the same subject without repositioning the sensors. Throughout the experiments (Test I and Test II for each subject), the average baseline (starting) fingertip temperature was 28·1 ± 3·8°C. The magnitude of the temperature fall during occlusion ($TF = T_{start} - T_{min}$) depends on the initial temperature and

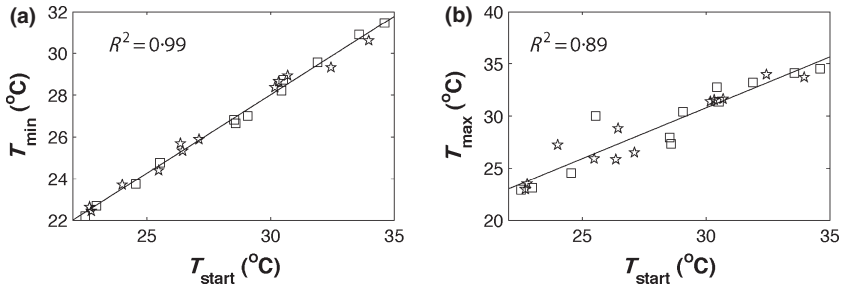

Figure 2 Linear relations between initial temperature (T_{start}) and both minimum temperature (T_{min}) and maximum temperature (T_{max}).

increases as the initial temperature increases. The temperature increase (TI) during postocclusion varied between 0·2 and 5·2°C and had an average value of 2·4 ± 1·6°C. The average temperature rebound or TR value was 0·9 ± 1·3°C with some subjects having starting temperatures below average showing significant rebound, while others showed negative TR values.

As shown in Fig. 2, T_{start} was strongly and linearly related to T_{min} (R^2 = 0·99) and T_{max} (R^2 = 0·89). These linear relations were also predicted by the mathematical model of Eq. (1). The relations observed in Fig. 2 provided a way of estimating T_{max} and T_{min} when the initial temperature is changed; particularly $T_{max} = (0{\cdot}97 \pm 0{\cdot}15)T_{start} + (1{\cdot}67 \pm 4{\cdot}26)$.

The relation observed between the LDF signal and fingertip temperature can be approximated by an exponential response, as illustrated in plots of the baseline perfusion (LDF_o) and starting temperature (T_{start}), as well as the relation between the maximum perfusion (LDF_{max}) and T_{max} (Fig. 3). The relations between the temperature parameters (T_{start}, T_{min}, T_{max} and TI) and Q_{max} follow a linear trend. As shown in Fig. 4, Q_{max} was significantly and positively associated with T_{max} (R^2 = 0·63) and LDF (R^2 = 0·36). The temperature signal shows a variation of the slope after occlusion is released, and the point at which the derivative changes was associated with the maximum Q. This illustrates the importance of heat flux or the time derivative of

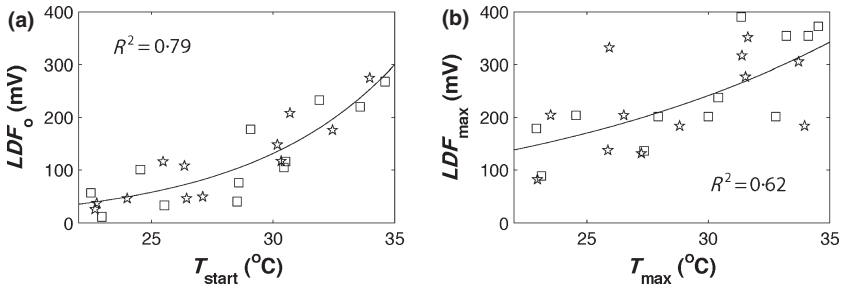

Figure 3 Relation between finger blood perfusion and the tissue temperature. Plot (a) indicates the correlation between basal perfusion (LDF_o) and the initial temperature (T_{start}). Plot (b) depicts the correlation between the maximum hyperaemic response (LDF_{max}) and the maximum temperature (T_{max}). The solid lines correspond to an exponential trend fitted using the least squares method. LDF, laser Doppler flowmetry.

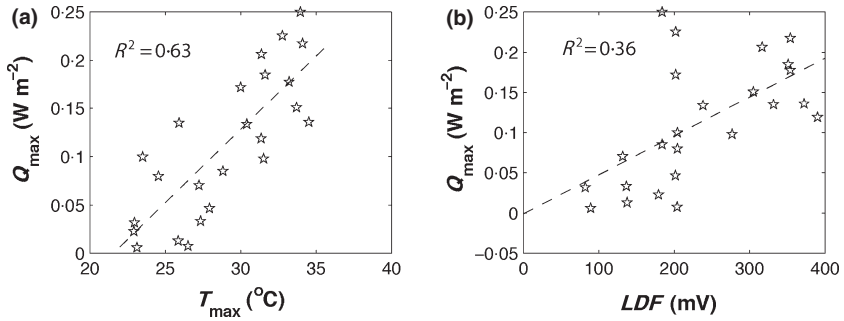

Figure 4 Relations between maximum heat flux (Q_{max}) recorded after occlusion (hyperaemia) and the maximum temperature (T_{max}) during digital thermal monitoring as well as laser Doppler flowmetry.

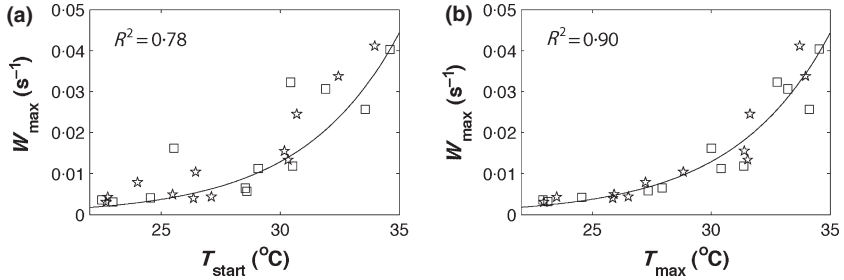

Figure 5 Relations between temperature parameters and the maximum perfusion function *W*. The perfusion function is calculated using the model provided in Eq. (2). The plots show an exponential relation between the maximum perfusion function (W_{max}) and both (a) the starting temperature (T_{start}) and (b) the maximum temperature (T_{max}).

the temperature signal during the early stages of postocclusion to estimate properties associated with the speed and magnitude of the hyperaemic response, parameters that are affected when vascular function is compromised (Tee *et al.*, 2004; Yvonne-Tee *et al.*, 2005).

The model of Eq. (2) together with the measured temperature and the subject-dependent H coefficient were used to estimate the perfusion function $W(t,T)$. The time derivative of the fingertip temperature of Eq. (2) was calculated numerically using a second-order accurate finite difference scheme. Figure 5 indicates the maximum value of the calculated hyperaemic function W_{max}, which follows an exponential trend with both T_{max} and T_{min}. The correlation observed for the calculated perfusion function W_{max} is significantly higher than the correlation of the LDF data (Fig. 3), which involves temperature and perfusion measured at different locations.

Discussion

The objective of the present study was to analyse the thermal response experienced by the fingers during brachial vascular reactivity tests and to correlate thermal parameters with flow parameters, including baseline perfusion, magnitude of the maximum hyperaemic perfusion and time to reach the hyperaemic peak (Yvonne-Tee *et al.*, 2005). The magnitude of the LDF signal was correlated with the local tissue temperature as an exponential behaviour between temperature and perfusion has been previously identified in different locations of the skin, such as the forearm and foot (Seifalian *et al.*, 1994; Allen *et al.*, 2002).

The temperature curves provide a smooth and robust response that clearly captures the moments of occlusion and reperfusion. The starting temperature (T_{start}) and the minimum temperature (T_{min}) have a significant effect over the thermal response during postocclusion, influencing the magnitude of the hyperaemic response. These experiments serve to establish relations between the magnitude of the hyperaemic response and the fingertip temperature recorded at the moment arterial occlusion is released. We found that the fingertip temperature follows the same response as that of perfusion or heat flux but shows a delay that is the result of anthropometric factors. As depicted in Fig. 2, the value of T_{min} and T_{max} depend strongly on the initial temperature (T_{start}). Our experiments indicate that when the starting temperature is higher than the upper bound of the thermoneutral skin temperature ($T_{start} > 33°C$), the postocclusive temperature increase (TI) and the rebound temperature (TR) are not robust as the vessels may already be maximally dilated. When initial fingertip temperature was close to that of the environment ($22°C < T_{start} < 24°C$), the LDF signal shows a smaller response and a more rapid decay.

As depicted in Fig. 1, the LDF signal exhibits rapid changes that are followed by heat flux and then temperature. The time delay between the LDF signal and fingertip temperature has been discussed in deep inspiratory gasp studies (Allen *et al.*, 2002). Deep inhalation (or gasp) causes a sudden characteristic change in blood flow in the finger pulp owing to vasoconstriction of the large number of arteriovenous shunts. The time required to restore normal flow is about 6·1 s. In healthy subjects, the flow reduction is followed by a small drop in fingertip temperature (0·08°C) that takes place within 29·1 s. The delay between the three signals (LDF, Q and T) represents the time required by the blood to warm up the finger tissue after occlusion is released. Such a time lag is a function of the system size (volume and surface area) as has been discussed in contact cooling studies (Kidd & Mc, 1958; Ozisik & Orlande, 2000) and its temperature at the moment reperfusion is restarted (T_{min}). It is observed that the time to peak measured from the heat flux signal ($t_{p,Q}$) is inversely proportional to the magnitude of the perfusion signal (LDF_{max}).

Heat flux measurements demonstrate rapid changes and follow variations in blood perfusion closely and respond more rapidly to changes in blood perfusion than temperature. The time at which the heat flux (or the derivative of the temperature signal) reaches a maximum corresponds to the time at which the temperature curves show an inflection point representing the moment at which the hyperaemic response is no longer the dominant factor determining the fingertip temperature. The reason that the maximum temperature is achieved later in time may be related to thermal inertia as reported in the inspiratory gasps studies (Allen *et al.*, 2002).

For obvious reasons, temperature and perfusion were recorded on different fingers in the present study, which could reduce the correlation between thermal and perfusion parameters. However, the measurements of fingertip temperature during the baseline period indicate that the same dynamic changes were observed in all fingers even though the fingertip temperature and heat flux were different in magnitude for each finger and along different locations of the same finger because of capillary bed distribution (Rosch *et al.*, 1977). This last observation is responsible for the well-known low reproducibility of LDF perfusion measurements as has been reported by several investigators (Savage & Brengelmann, 1994; Cracowski *et al.*, 2006).

There are several limitations that should be emphasized. First, the sample size used in the present study is relatively small. Second, all the subjects studied were young and apparently healthy, and it is not clear whether the same relations can be obtained in older or patient populations.

The experiments performed herein allowed us to test two responses in the same subject, without the need of repositioning of the sensors and therefore removing the effects of sensor placement on the repeatability of thermal behaviour. For successful characterization of the hyperaemic response using temperature, the starting (baseline) temperature should be between 28 and 33°C. This temperature range will ensure a linear relation between perfusion and skin temperature. To avoid the contribution of initial vasoconstriction or vasodilatation, arterial occlusion should not be started until the fingertip temperature is stabilized. Future experiments are needed to investigate the repeatability of the response, which has been observed to be strongly dependent on the initial or starting temperature.

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