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Pulse transit times to the capillary bed evaluated by laser Doppler flowmetry

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Abstract

The pulse transit time (PTT) of a wave over a specified distance along a blood vessel provides a simple non-invasive index that can be used for the evaluation of arterial distensibility. Current methods of measuring the PTT determine the propagation times of pulses only in the larger arteries. We have evaluated the pulse arrival time (PAT) to the capillary bed, through the microcirculation, and have investigated its relationship to the arterial PAT to a fingertip. To do so, we detected cardiac-induced pulse waves in skin microcirculation using laser Doppler flowmetry (LDF). Using the ECG as a reference, PATs to the microcirculation were measured on the four extremities of 108 healthy subjects. Simultaneously, PATs to the radial artery of the left index finger were obtained from blood pressure recordings using a piezoelectric sensor. Both PATs correlate in similar ways with heart rate and age. That to the microcirculation is shown to be sensitive to local changes in skin perfusion induced by cooling. We introduce a measure for the PTT through the microcirculation. We conclude that a combination of LDF and pressure measurements enables simultaneous characterization of the states of the macro and microvasculature. Information about the microcirculation, including an assessment of endothelial function, may be obtained from the responses to perturbations in skin perfusion, such as temperature stress or vasoactive substances.

Keywords

pulse transit time; laser Doppler flowmetry; arterial stiffness; pulse wave velocity; microcirculation; vascular aging; endothelium

1. Introduction

Measurement of the propagation times of cardiac-induced pulse waves along the arterial tree provides an important tool for studying arteries. It enables their viscoelastic properties to be quantified in terms of arterial stiffness or its inverse, arterial compliance. The arterial stiffness is being used increasingly in clinical assessment and diagnosis on account of its role in the development of cardiovascular disease (Laurent *et al* 2001, 2006).

The pulse transit time (PTT) is defined as the time taken for the pulse waveform generated by the heart to traverse a certain section of artery. It is a simple, reproducible and non-invasive measure. Knowing the propagation distance, one can use the PTT to calculate the pulse wave velocity, which is generally accepted as being the most robust of the available

indices of arterial stiffness (Laurent *et al* 2006, Hirata *et al* 2006). Apart from the calculation of pulse velocity, PTT measurements have also been used recently as an indirect estimate of blood pressure changes in healthy subjects (Foo *et al* 2006); for the non-invasive detection of rapid changes, such as during obstetric spinal anaesthesia (Sharwood-Smith *et al* 2006) or during hemodialysis (Ahlstrom *et al* 2005).

Tounian *et al* (2001) showed a correlation between absolute arterial stiffness and the endothelial function. Maltz and Budinger (2005) showed how changes in PTT can be used to measure the smooth muscle relaxation that occurs when a functioning endothelium is stimulated. Studies in children have related arterial stiffness directly to impaired endothelial function, for example, in cases of low birth weight (Martin *et al* 2000) or severe obesity (Tounian *et al* 2001). Several lines of evidence support the hypothesis that endothelium regulates arterial stiffness by release of vasoactive mediators. Inhibition of basal nitric oxide (NO) production in the endothelium with L-NMMA, for example, increases the stiffness of the brachial artery (Kinlay *et al* 2001). Acetylcholine (ACh), an endothelium-dependent vasodilator, reduces stiffness in large arteries, but the reduction is inhibited by L-NMMA, an inhibitor of NO synthase (Wilkinson *et al* 2002).

The measurement of pulse wave velocity or PTT can be performed automatically (Asmar *et al* 1995), but there are several approaches to the choice of measurement site and the methodology of pulse detection (Laurent *et al* 2006). Common methods for the evaluation of PTT are based on detecting blood pressure pulses, blood velocity pulses, or volume changes in the arteries by applying Doppler ultrasound, applanation tonometry or photoplethysmography (Lehmann *et al* 1993, Davies and Struthers 2003, Allen 2007).

Most clinically relevant techniques in the field are limited to the detection of stiffness in large arteries. It has recently been shown that using photoplethysmography, PTT to the small peripheral arteries can be used to assess the stiffness of the lower body arteries (Babchenko *et al* 2000, Nitzan *et al* 2002). These transit times include the time for the pulse to travel through the aorta, large peripheral arteries and small arteries in the tip of the finger or toe. However, the propagation of pulse waves through the network of capillaries has not been investigated. In the microcirculation, the pulsation of the heart is reduced compared to that in the arteries, so that the detection of pulse waveforms is not straightforward. A suitable method for this purpose is laser Doppler flowmetry (LDF), which enables non-invasive and continuous measurements of microvascular perfusion (Nilsson *et al* 1980, Humeau *et al* 2007) and is widely used in the assessment of the dynamical properties of skin microcirculation, both in health and in various cardiovascular diseases (Rossi *et al* 2006, Ažman-Juvan *et al* 2008). As far as we are aware, LDF has not yet been used to detect pulse waves in the microcirculation with the purpose of obtaining pulse transit times.

The aim of the current study was to demonstrate a method for the measurement of delays in cardiac-related pulses in the skin microcirculation, using LDF. The pulse arrival time delays to the microcirculation (PAT_m) on the four extremities were determined, using the R-peak in the ECG as a reference. The results were compared with pulse arrival times to the small peripheral arteries in the fingertip (PAT_f), detected from a simultaneously measured blood pressure signal. The difference between these two arrival times is introduced for the first time as a measure of the pulse transit time through the microcirculation (PTT_{mic}), for the purpose of obtaining information about the state of the microvasculature.

2. Methods

2.1. Subjects

The study included 108 healthy subjects with no history of cardiovascular disease: 64 males and 44 females. Their mean age with standard deviation was 50 ± 16 (range 15–84) years. Their mean systolic blood pressure (SBP) was 127 ± 17 mmHg, diastolic pressure (DBP) was 78 ± 9 mmHg and heart rate (HR) was 64.2 ± 8.4 beats per minute.

All participants gave their informed consent, and the study was approved by the local ethics committee.

2.2. Experimental protocol

Simultaneous measurements of cardiovascular signals were recorded from each subject while lying in a supine position. Using a signal-conditioning system for physiological signals (Cardiosignals, Institute Jožef Stefan, Slovenia) and an LDF monitor (floLAB, Moor Instruments Ltd, UK), continuous time series of ECG, blood pressure and four channels of laser-Doppler blood perfusion were recorded for 30 min as shown in figure 1. The ECG was measured at a 1000 Hz sampling frequency with the electrodes attached to both shoulders and the lowest left rib. Clear R-peaks were obtained in the signal (figure 2(a)) and their positions are used as markers for the beginning of ventricular ejection and initiation of the pulse wave in the arteries. A piezoelectric sensor (MLT1010/D Piezo Electric Pulse Transducer, ADInstruments) was attached to the tip of the left index finger (figure 2(d)) to measure blood pressure at 400 Hz in the radialis indicis artery, a branch of the radial artery that provides blood to the index finger. The sensor measures dynamical changes in pressure, and its output has to be integrated to obtain time series of blood pressure. After integration, the maxima and minima of the resultant signal represent the systolic and diastolic pressures. They were shown to be at positions identical to those obtained with an infrared sensor (Paluš *et al* 2004). The arrival time of the pressure pulse was determined from the minimum of the curve at the end of the diastole, i.e. the foot of the systolic increase. Using the R-peak in the ECG as a reference, the pulse arrival time to the foot of the corresponding pressure wave (PAT_f) was calculated (figure 2(b)).

Microvascular blood perfusion signals were recorded on the four extremities (right arm: RA, left arm: LA, right leg: RL and left leg: LL) using the LDF monitor. To standardize the recording sites and avoid subcutaneous arteries, skin over the bony prominences on the wrist (left and right caput ulnae) and inner side of the ankle (left and right medial malleolus) was chosen (figure 1), and the probes (MP1-V2, Moor Instruments Ltd, UK) were attached to the skin surface with double-sided adhesive discs. Note that in contrast to pressure measurements from the arteries in the finger, LDF signals are detected from areas with no big vessels present. The evaluation of perfusion in the microcirculation is based on the Doppler effect when the photons from the coherent laser light interact with the moving red blood cells in the capillary bed (Nilsson *et al* 1980, Humeau *et al* 2007). The term commonly used to describe LDF recordings is ‘flux’, a quantity proportional to the product of average speed of the blood cells and their number concentration (often referred to as blood volume). It is expressed in arbitrary units (AU). The wavelength of the light was 780 nm, the cut-off frequency of the low-pass filter was set to 22.5 kHz and an output time constant of 0.1 s was selected on the monitor. Signals were digitized at a sampling rate of 40 Hz using a 16-bit A/D converter (PCI-6035E, National Instruments). Note that throughout the study statistical data are focused mainly on LDF recordings from the LA, which is the arm on which the pressure is also measured. Data from the other extremities are presented for investigation of spatial reproducibility.

Wavelet-based time–frequency transformation of LDF time series from blood flow has revealed that they consist of several periodic components spanning a relatively wide frequency range (Stefanovska *et al* 1999). Cardiac activity and respiration are the origins of oscillations with the highest two frequencies. Both propagate along the cardiovascular system and appear to be strongly frequency and phase synchronized when observed at different sites (Stefanovska and Hoži 2000).

To extract the cardiac-related component from the perfusion signals, band-pass filtering was performed using a fourth-order Butterworth filter with zero-phase distortion. Due to the inter-subject heart rate variations, the middle of the passband was set to the mean frequency of the heartbeat, and the cut-off frequencies were set to 0.2 Hz below and above the mean value. The heart rate for each subject also varies in time. These variations were accommodated by choosing the Butterworth filter with its maximally flat amplitude response in the passband. Maxima of the filtered signals were chosen as markers for the localization of the pulses. The arrival times of the pulses to the peripheral microcirculation (PAT_m), relative to the R-peaks in the ECG were defined as shown in figure 2(c). Note that only the PAT_m to the left arm is presented in the figure.

To verify that the PAT_m to the arms and legs is shorter than one heartbeat period, series of inter-beat values were constructed from the positions of R-peaks and filtered perfusion maxima. Their cross correlation revealed a delay of about 0.5 s, showing that the arrival of the pulse waveform to the microcirculation on both extremities precedes the next heartbeat.

Propagation times between each R-peak and their corresponding pressure and perfusion pulse waveforms on the extremities were obtained over the 30 min of each recording. PAT_f and PAT_m for each subject were calculated as their mean value over the whole recording. Depending on the subjects' heart rate, this averaging included approximately 1800 pulse arrival times for each quantity.

The locations of markers, such as the R-peaks in the ECG, foot of pressure waves and maxima of filtered perfusion signals were automatically detected and manually checked to correct any discrepancies.

The analysis in the present work was performed using algorithms written within the Matlab (The MathWorks, Inc.) environment and using standard functions from Matlab's toolboxes.

2.3. Cooling experiment

A separate experiment was performed on eight healthy subjects (two females and six males, aged 24–64 years, mean 38 years) to test the sensitivity of PAT_m to local cooling of the skin and its consequent effect on the microcirculation. For a continuous period of 80 min, perfusion data and skin temperature were recorded with the LDF probe and the temperature sensor (Thermilinear, YSI Incorporated, OH) placed close together on the volar aspect of the forearm. After 30 min of basal recording, local cooling was applied to the skin. An ice pack was applied to the measurement area and its immediate vicinity, and an intervening layer of fabric was used to lessen the thermal shock. After 20 min, the ice pack was removed while the measurement continued for another 30 min. To investigate the effect of local cooling, PAT_m was calculated by band-pass filtering the perfusion signal as described in the previous section. For each heartbeat cycle, the pulse arrival time to the skin microcirculation was obtained, relative to the corresponding R-peak. Time series of arrival time values were constructed for the whole duration of the recording to observe the effect of cooling. Note that the site of recording in this experiment was on the volar aspect of the arm, which represents a difference from the rest of the study where bony prominences were used.

Data from this experiment were previously presented in the investigation of phase coherence between oscillations in LDF signals and skin temperature (Bandrivskyy *et al* 2004).

2.4. Statistical analysis

The relationship between transit times and physiological parameters such as age, HR, SBP and DBP was examined by correlation analysis and linear regression. Spearman's rank correlation coefficient (r_s) was calculated, and the statistical significance of dependence was evaluated by the calculation of the p -value. The PAT_m values from the four extremities were compared by applying a paired Wilcoxon signed-rank test. In all statistical tests $p < 0.05$ was considered as statistically significant. Statistical analysis was performed using the Matlab Statistics Toolbox.

3. Results

3.1. PAT_f and PAT_m

The mean value and standard deviation of PAT_f for all subjects is 0.18 ± 0.03 s, which is in agreement with the results of Nitzan *et al* (2002). Mean values and standard deviations of PAT_m are given in table 1. There is no statistically significant difference between arrival times to the two arms ($p = 0.74$) and between arrival times to the two legs ($p = 0.11$). Between the LA and LL there is a significant difference ($p = 0$), while for the right side (RA compared to RL) a difference is indicated but the p -value is on the border of significance level ($p = 0.07$).

The mean values of PAT_m (table 1) are much higher than PAT_f for two reasons. First, the arrival time detected by LDF includes the propagation of the pulse wave from the small peripheral arteries to the capillary bed. Second, the maxima of the filtered perfusion signal, which were considered as markers, necessarily occur later than the foot of the pressure wave as seen in figure 2.

There is a statistically significant negative correlation between PAT_f and age ($r_s = -0.43$, $p = 0$), and between PAT_f and heart rate ($r_s = -0.30$, $p = 0$) as shown in figures 3(a) and (b). The relationship with age and HR was also investigated for PAT_m from all four extremities.

PAT_m from the LA (PAT_m^{LA}) is negatively correlated with both age ($r_s = -0.27$, $p = 0.02$) and HR ($r_s = -0.63$, $p = 0$), as seen in figures 3(c) and (d). Similarly from the RA, statistically significant negative correlations between PAT_m and age ($r_s = -0.24$, $p = 0.02$), and between PAT_m and HR ($r_s = -0.55$, $p = 0$) are revealed. For both legs PAT_m is negatively correlated with HR (RL: $r_s = -0.55$, $p = 0$ and LL: $r_s = -0.54$, $p = 0$) while the relationship with age is negative but not significant (RL: $r_s = -0.11$, $p = 0.29$ and LL: $r_s = -0.16$, $p = 0.15$).

PAT_m data in figure 3 are presented only for the LA, for comparison with PAT_f data from pressure recordings on the same arm. Note that the negative correlation between the pulse arrival times and HR is stronger for all four PAT_m compared to PAT_f . The standard deviations of arrival times (figures 3(a), (c)) and heart rates (figures 3(b), (d)) are presented as full lines, extending from the mean value, which is marked with a dot. The relatively high standard deviations of arrival times are on account of averaging them over the whole 30 min recording, which includes approximately 1800 PAT values.

A statistically significant negative correlation is obtained between PAT_f and SBP ($r_s = -0.26$, $p = 0.02$) and between PAT_f and DBP ($r_s = -0.41$, $p = 0$) presented in figures 4(a) and (b), but not between any PAT_m and SBP (RA: $r_s = -0.13$, $p = 0.23$, LA: $r_s = -0.02$, $p = 0.86$, RL: $r_s = -0.23$, $p = 0.05$ and LL: $r_s = -0.18$, $p = 0.10$) or between any PAT_m and DBP

(RA: $r_s = -0.13$, $p = 0.23$, LA: $r_s = -0.17$, $p = 0.13$, RL: $r_s = -0.20$, $p = 0.06$ and LL: $r_s = -0.15$, $p = 0.17$).

The subjects' SBP is positively correlated with their age ($r_s = 0.41$, $p = 0$), but there is no correlation between HR and age ($r_s = 0.08$, $p = 0.40$) or between HR and SBP ($r_s = 0.17$, $p = 0.08$).

The arrival time, PAT_f , is positively correlated with PAT_m from all extremities, and all correlations are statistically significant. The highest correlation is between PAT_f and PAT_m from the LA ($r_s = 0.57$, $p = 0$) both obtained from the same extremity. The correlation coefficients for PAT_m from the other extremities are lower (RA: $r_s = 0.28$, $p = 0.02$, RL: $r_s = 0.31$, $p = 0.01$ and LL: $r_s = 0.28$, $p = 0.03$).

3.2. PTT through the microcirculation (PTT_{mic})

Two arrival times were detected from the left arm: PAT_f to the peripheral arteries in the index finger, and PAT_m^{LA} to the skin microcirculation on the wrist. Their difference is introduced as a measure of the pulse transit time through the microcirculation. It was calculated as $PTT_{mic} = PAT_m^{LA} - PAT_f$.

There is a significant negative correlation between the transit time through the microcirculation and the heart rate ($r_s = -0.53$, $p = 0$) as shown in figure 5. The relationships with age ($r_s = 0.03$, $p = 0.81$), SBP ($r_s = 0.19$, $p = 0.16$) and DBP ($r_s = -0.07$, $p = 0.62$) are not significant.

3.3. PAT_m and cooling of the skin

A typical continuous response to cooling of the skin for one subject is presented in figure 6. During cooling through about 6 °C, the skin temperature decreased exponentially, and then recovered towards the initial value during the last 30 min after the cold pack had been removed (panel (a)).

The mean perfusion and the amplitude of its oscillations suddenly increased at the beginning of cooling, and then decreased continuously (panel (b)). A slight decrease of the heart rate with time is obtained throughout the recording (panel (c)), with no obvious correlation with the cooling period. PAT_m for the whole recording was calculated and is presented in panel (d). During cooling, a continuous decrease of mean PAT_m and its variability was observed. After the cooling period the variation of PAT_m increased and its mean value had a slightly increasing trend.

The data from all eight subjects are summarized in table 2. Their maximum skin temperature before cooling, minimum temperature during cooling and maximum temperature after cooling are tabulated. For each period, the mean PAT_m and its standard deviation are given. In all subjects the mean PAT_m is decreased during the cooling period. In five subjects, PAT_m increased at the 80 min time point, 30 min after the ice pack was removed. In the other three subjects, either no change or a slight decrease in PAT_m was observed.

3.4. PAT_m values in time

With the exception of the cooling experiment, all PAT_m data in the study are given as mean values over the whole 30 min recording. Here we describe how the arrival times changed throughout the measurement. Figure 7 presents the PAT_m time series from all four extremities of one subject. Note that the mean PAT_m to the legs is slightly higher than to the arms, as summarized in table 1.

No changes of mean PAT_m values or their variations in time can be detected by visual inspection. Mean values and standard deviations of PAT_m time series for the first 15 min (0–15 min) and the second 15 min (15–30 min) of the recording were compared and the statistics using a paired Wilcoxon signed-rank test is presented in table 3. The mean values of PAT_m in the second half are significantly higher than in the first 15 min for all extremities. On average, PAT_m from the RA increased by 2%, from the LA by 8% and from both legs by $\approx 0.5\%$.

Standard deviations did not change for the LA, RL or LL. In the RA, the standard deviation of PAT_m significantly decreased in the second 15 min ($p < 0.01$). Using a non-paired test (Wilcoxon rank-sum test), no changes in either mean values or standard deviations were obtained.

Correlations between mean values of PAT_m from the first half, and mean values from the second half, of the recordings are presented in figure 8. For all extremities a high correlation index was obtained, $r_s \approx 0.96$.

4. Discussion

4.1. Methodological issues

The arrival of the pressure pulse to the peripheral artery can be specified in terms of some characteristic point in the pressure waveform. Several possibilities have been previously proposed, but there is no consensus for a definitive method (O'Rourke *et al* 2002). In the present study, the foot of the wave was chosen because it is least likely to be distorted during its forward propagation through the arterial tree (Chiu *et al* 1991, O'Rourke and Hayward 2003). The locations of the systolic pressure (pressure wave maxima) were tested as an alternative option, but in this case none of the physiological parameters were correlated with the transit time.

The perfusion signal does not, however, exhibit the same waveform as the pressure, and the foot of the wave is not straightforward to locate. Therefore, minima and maxima of the filtered perfusion signals were tested as possible markers, and both were found to give similar results in terms of statistical analysis. Occasional discrepancy between the original and filtered perfusion signal at the minimal values produces an incorrect and unrealistically low PAT_m value. This results in increased time variability of PAT_m when minima are used as markers. Therefore, maxima of the filtered perfusion signals were preferred to locate the pulse wave in the microcirculation. A compromise between physiological relevance and numerical reliability was thus achieved.

By defining the initiation of pulse propagation to be from the R-peak in the ECG signal, the time delay between the R-peak and the opening of the aortic valve (pre-ejection period, PEP) is added to the arrival times. The PEP was not measured in the present study, and therefore the absolute values of arrival times have not been designated as PTT measurements.

Pulse waves in the pressure signal are localized in time by the foot of the waveform, whereas pulses in the perfusion are localized by their maxima. For this reason, direct comparison of absolute PAT_f and PAT_m values has not been performed. However, comparison of their relation to physiological parameters can provide qualitative information. This can be justified by the fact that both transit times include the same PEP, and the propagation along the arterial pathway is identical. The only difference is related to the propagation of the pulse wave through the microcirculation.

A comparison of PAT_m values from the four extremities reveals a symmetry between the right and the left sides of the body for both arms and legs. Furthermore, the right–left symmetry is also obtained from correlations with physiological parameters. In the group of healthy subjects, this is an expected finding which demonstrates the spatial reproducibility of the method.

PAT_m data in the present study are obtained from 30 min recordings of cardiovascular signals. The acquisition time of most current PTT detection methods usually includes no more than a few cardiac cycles. Because of intrinsic fluctuations of the heart rate, the stroke volume and other cardiovascular parameters, averaging over longer time windows is necessary to provide more reliable estimation of the PAT_m . We do not discuss the question of an optimal time window over which the averaging is performed. Nevertheless, based on previous studies of oscillations and fluctuations in LDF and other cardiovascular signals (Stefanovska *et al* 1999, Stefanovska and Hoži 2000), we propose at least several minutes of recording. Longer recording can introduce some drift in the value of the estimated PAT_m . Indeed, a comparison of averages over the first 15 min and the second 15 min demonstrated a small but systematic increase in PAT_m which varied from 0.5% on the legs to 8% on the left arm.

4.2. PAT_f , PAT_m and physiological parameters

The results of the study show a decreased PAT_f with age, heart rate, and systolic and diastolic blood pressures. Age and systolic pressure were previously shown to be associated with increased pulse wave velocity in both central (Asmar *et al* 1995) and peripheral arteries (Nitzan *et al* 2002). The relationships between pulse wave velocity and HR were previously investigated during cardiac pacing, and a positive correlation has been reported (Drinnan *et al* 2001, Lantelme *et al* 2002, Millasseau *et al* 2005). However, the underlying mechanisms remain poorly understood. The present study reveals a decreased PAT_f with HR, which is unrelated to the subject's age or SBP. The PAT_f measurements include PEP, which was not measured, so that the possible effect of PEP on the HR cannot be addressed. The observed negative correlation between PAT_f and DBP was reported previously, with a significant decrease of PAT_f with DBP for DBP > 85 mmHg (Babchenko *et al* 2000), but not for DBP < 85 mmHg (Babchenko *et al* 2000, Nitzan *et al* 2002).

With detection of pulse waves by LDF, the propagation time through the capillaries is included within the arrival time. The correlations with physiological parameters reveal similar results as with PAT_f : a negative correlation of PAT_m with age on both arms and a negative correlation with HR on all extremities. Lack of correlation between PAT_m and both pressures (SBP and DBP) is probably on account of the drop in blood pressure on the level of terminal arterioles and capillaries, where the LDF signal is measured. The agreement between the two arrival times clearly demonstrates the usefulness of the LDF method for detection of cardiac-related pulse waves and its application to obtain transit times from the microcirculation.

4.3. PAT_m and local cooling

During the cooling of the skin we have observed a clear decrease of PAT_m in all subjects. A $\approx 6^\circ\text{C}$ drop in skin temperature decreased the mean PAT_m by more than 0.05 s. This is a relatively large change considering that PAT_f is ≈ 0.18 s. The effect of local cooling on the PAT_f , detected from the pressure pulses in the peripheral arteries of the fingertip was previously examined (Zhang and Zhang 2006). In the latter study, the finger was cooled for $\approx 6^\circ\text{C}$ but no effect of cooling on pulse transit time (equivalent to PAT_f in the present study) was found compared to the reference finger. This suggests that in the current study, the changes in PAT_m during cooling mainly originate from faster propagation of pulses through

the microcirculation. Therefore, the LDF method adds additional value to the PAT compared to arterial pressure pulse based measurements and can provide information about the state of the microvasculature.

Exposure of the skin to cold stress resulted in cutaneous vasoconstriction due to the several local and neural mechanisms (Kellogg 2006). Consequently, the blood flow in the skin is reduced. The reduction in blood flow on the volar aspect of the forearm is not proportional to the temperature decrease, but becomes more rapid at temperatures above 31 °C (Vuksanovi *et al* 2008). In the present study, a sharp increase in the LDF signal is obtained at the beginning of cooling. It is probably related more to a slightly changed position of the LDF probe than to the effect of cooling. With application of the ice pack, the flexible probe holders let the probe closer to the surface of the skin. Due to high sensitivity of the LDF signal to the position of the probe, the reduction in perfusion is not observed.

In contrast to the case of cooling, heating of the skin resulted in vasodilation and increased blood flow (Kellogg 2006, Vuksanovi *et al* 2008). In response to decreased tonus of the smooth muscles in the measurement area, a slower propagation of the pulse waves and an increase in PAT_m is expected. For detailed investigations of pulse propagations in the microvasculature in response to temperature stress however, further work will be needed, including a methodology with controllable temperature stress and comparison with control measurements.

4.4. PTT through the microcirculation

From the relationship between transit times and HR, it was found that there is a stronger correlation between HR and PAT_m on all extremities compared to PAT_f . Moreover, the correlation with the HR is preserved when the transit time through the microcirculation only (PTT_{mic}), as obtained from the difference between PAT_m from the LA and PAT_f , is considered. Whether this is due to the difference between the LDF and pressure measurement techniques, or due to the inclusion of capillary transit time when LDF is applied, is beyond the scope of this study.

The dynamics of the skin perfusion, detected by LDF, consists of several oscillatory components (Stefanovska *et al* 1999). The continuously changing tonus of surrounding smooth musculature modulates the propagation time in the microcirculation. The oscillations in LDF with the two lowest characteristic frequencies were shown to reflect endothelial activity. Their origin was investigated in response to vasoactive substances (Kvandal *et al* 2003, 2006). Endothelium-dependent vasodilation due to ACh decreases the stiffness of large arteries (Wilkinson *et al* 2002) and it is expected that in the microcirculation, consisting of capillaries each made up of a single layer of endothelial tissue, the same effect will occur. Indeed, a several-fold increase in average blood flow and in all oscillatory components can be achieved by iontophoretically administered ACh, resulting in muscle relaxation. Continuous measurements of PTT through the microcirculation in response to vasodilatory substances might therefore provide a possible method to assess the endothelial function in the microvasculature.

While ACh induces vasodilation, anaesthesia appears to elicit the opposite effect, leading to a reduction in the perfusion as measured by LDF. Low-frequency oscillations are dramatically decreased during both local (Landsverk *et al* 2006) and global anaesthesia (Landsverk *et al* 2007). Whether this changes the transit time significantly in the microcirculation remains to be investigated.

In addition to perturbations of the perfusion with temperature stress, vasodilator substances or anaesthesia, transit times through the microcirculation can be investigated in various

cardiovascular diseases. With the introduction of PTT_{mic} we propose a new index that may have important clinical relevance.

5. Conclusion

We have introduced LDF as a method for quantifying pulse wave timing in the skin microcirculation, calculating PAT_m from the ECG R-peak as a reference. Our results are in agreement with PAT_f calculated from peripheral pressure wave measurements, indicating the usefulness of LDF in tracing the propagation of pulses in the microcirculation. With transit times through the capillaries included within the arrival time, information about the state of microcirculation can be obtained. Furthermore, using a combination of pressure wave measurements and LDF, we have introduced a measure for pulse transit time through the microcirculation, PTT_{mic} . The method has potential for the continuous measurement of transit times and for investigations of their responses to perturbations in the skin using temperature stress, during anaesthesia, or in various cardiovascular diseases. Responses to vasoactive substances might provide information about endothelial function in the capillary bed. Many recent studies have illuminated the involvement of endothelium and its dysfunction in aging, and in diseases such as hypertension and diabetes. Transit times evaluated from LDF recordings have important potential for clinical applications either as a stand-alone method or in combination with pressure recording.

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Figure 1. Schematic diagram of the measurement setup. Simultaneous measurements of cardiovascular signals were recorded for 30 min and included ECG, blood pressure and four channels of LDF signals, one from each extremity: right arm (RA), left arm (LA), right leg (RL) and left leg (LL).

Figure 2.

The definition of pulse arrival times, PAT_f and PAT_m , demonstrated on short segments of simultaneously recorded cardiovascular signals: (a) ECG, (b) blood pressure and (c) LDF signal. Marker points were detected and the pulse arrival times were calculated from the R-peak. In (d), the measurement sites on the tip of the left finger and bony prominence on the wrist are depicted. Pressure is detected from the arteries while LDF is measured over areas with no big vessels present. Only LDF recordings from the left arm are presented in the figure.

Figure 3.

Correlations between pulse arrival times PAT_f and PAT_m from the left arm (PAT_m^{LA}) and heart rate (HR) and age. All correlations are statistically significant ($p < 0.05$) and the Spearman's rank correlation coefficients (r_s) are presented. For each subject, the mean PAT values are shown as dots and their standard deviations as solid lines extending from the mean. The relatively high variations, presented for (a) and (c) arrival times, and for (b) and (d) HR, are on account of averaging over approximately 1800 PAT values.

Figure 4. Correlations between (a) PAT_f and SBP, and (b) PAT_f and DBP. Both correlations are statistically significant ($p < 0.05$) and the Spearman's rank correlation coefficients (r_s) are given. The transit time data are calculated as averages over the 30 min recordings, including approximately 1800 PAT_f values.

Figure 5.

The PTT through the microcirculation (PTT_{mic}) is evaluated as the difference between PAT_m^{LA} and PAT_f , both measured on the left arm. The transit time is negatively correlated with the HR. The Spearman's rank correlation coefficient (r_s) is given.

Figure 6.

The effect of local cooling on PAT_m , calculated from the LDF signal on the volar aspect of the forearm. Signals of (a) skin temperature and (b) LDF perfusion were measured, and the (c) HR and (d) PAT_m values were calculated for the whole duration of the experiment. Cooling was applied for 20 min in the middle of the 80 min recording, as indicated by the vertical dashed lines.

Figure 7.

PAT_m values and their variations in time, presented for one subject. They are calculated from LDF signals, measured on the (a) RA, (b) LA, (c) RL and (d) LL. Time series are constructed from arrival time values, calculated from each R-peak in a 30 min recording and its corresponding pulse wave.

Figure 8.

Correlation between mean PAT_m values from the first 15 min (0–15 min) and second halves (15–30 min) of the recording period. The Spearman's correlation coefficient (r_s) is displayed for PAT_m from the (a) RA, (b) LA, (c) RL and (d) LL.

Table 1

Statistics of PAT_m values for all subjects, calculated from the four extremities

	PAT_m (s) Mean $\pm$ SD	<i>p</i> -value
RA	0.568 ± 0.094	0.74
LA	0.551 ± 0.080	
RL	0.585 ± 0.068	0.11
LL	0.588 ± 0.063	

Table 2

Mean values and standard deviations of PAT_m before, during and after cooling for eight subjects. The maximum skin temperature before cooling, the minimum temperature during cooling and the maximum temperature after cooling are also given

Subject no		Basal	Cooling	Basal
Subject 1	PAT_m (s)	0.616 ± 0.106	0.452 ± 0.052	0.452 ± 0.099
	temp ($^{\circ}C$)	32.8	26.3	31.2
Subject 2	PAT_m (s)	0.623 ± 0.098	0.491 ± 0.086	0.478 ± 0.122
	temp ($^{\circ}C$)	31.6	24.6	29.0
Subject 3	PAT_m (s)	0.499 ± 0.029	0.439 ± 0.037	0.450 ± 0.041
	temp ($^{\circ}C$)	33.5	27.1	30.6
Subject 4	PAT_m (s)	0.596 ± 0.045	0.490 ± 0.084	0.524 ± 0.104
	temp ($^{\circ}C$)	33.3	28.0	31.3
Subject 5	PAT_m (s)	0.594 ± 0.05	0.563 ± 0.099	0.583 ± 0.099
	temp ($^{\circ}C$)	34.0	30.1	32.5
Subject 6	PAT_m (s)	0.421 ± 0.052	0.403 ± 0.044	0.444 ± 0.059
	temp ($^{\circ}C$)	33.3	29.6	33.1
Subject 7	PAT_m (s)	0.455 ± 0.029	0.430 ± 0.036	0.415 ± 0.041
	temp ($^{\circ}C$)	34.5	29.9	33.6
Subject 8	PAT_m (s)	0.529 ± 0.131	0.375 ± 0.085	0.594 ± 0.140
	temp ($^{\circ}C$)	34.8	30.5	34.1

Table 3

Comparison of mean PAT_m values and their standard deviations from the first 15 min and second 15 min recording using a paired Wilcoxon signed-rank test

	PAT_m			
	RA	LA	RL	LL
Mean $\langle 0-15 \text{ min} \rangle$ versus $\langle 15-30 \text{ min} \rangle$	$p = 0$	$p = 0$	$p = 0$	$p = 0$
SD $\langle 0-15 \text{ min} \rangle$ versus $\langle 15-30 \text{ min} \rangle$	$p = 0$	$p = 0.57$	$p = 0.34$	$p = 0.61$