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Rhythmical Variations of Haemoglobin Oxygenation in Cutaneous Capillaries

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The consequences of rhythmical arteriolar vasomotion for nutrition and the tissue oxygen supply to human skin are largely unknown. In the study presented here, the periodic variations of haemoglobin oxygenation in the small cutaneous vessels have been evaluated with a new reflection spectrophotometer. For the assessment of spatial variations, we examined 24 different sites in 20 healthy volunteers. For quantification of the relatively long duration of periodic variations, a Digital Fourier Transformation with a specially programmed filter was used.

In 265 out of 480 spectra (55.2%), periodic variations of the haemoglobin oxygenation were found. The average of the main frequency of waves was 7.0 ± 2.5 cycles per minute. The occurrence of variations of haemoglobin oxygenation depended on the measuring site. In the gluteal region, variations were observed in 17 out of 20 subjects, on the palms in 16 out of 20, at the foot plantar in 18 out of 20 in comparison to the cheek (8/20), the lip (5/20) and the eyelid (6/20). On the head we observed significantly more variations per minute than in the lower extremities.

Because these variations, with duration of up to 30 s, have a relatively slow dynamic compared with heart rate and breathing frequency, consequences for the cutaneous diffusion and metabolism of other substrates are very likely. ***Key words: Digital Fourier Transformation; vasomotion; microcirculation; skin oxygen supply.***

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Rhythmic arteriolar vasomotion is a well-known physiological phenomenon found in different species and in different tissues. For assessing those temporal variations in blood flow in the arteriovenous subpapillary plexus, laser Doppler flowmetry can be used (1). Whereas this technique gives information about the haemodynamic occurrences in the area of arteriovenous shunts (HeNe with 632.8-nm wavelength), we know little about the consequences of vasomotion on the oxygen supply to the skin. An important parameter of tissue oxygen supply is the oxygen saturation of haemoglobin, which can be assessed with spectrophotometry.

Reflection-optical methods use different ranges of wavelengths. Reflection pulse oximetry (2) and near infrared spectroscopy (NIR) apply wavelengths which have light penetration depths of some millimetres (3, 4). Therefore, using these techniques, not only the dermis is measured, but also the underlying tissue. Using reflection spectrophotometric methods (5) operating on visible wavelengths (ca. 500–630 nm), the penetration depth is lower (1–2 mm (6)) and the skin can be monitored. However, these methods have an important disadvantage over NIR, as the light path, and therefore the reflection spectrum, is disturbed at shorter wavelengths by absorption and scattering in the skin (2, 3). To separate the haemoglobin reflection spectrum and skin scattering in a multicomponent spectrum, the two-flux theory of Kubelka-Munk is often used (7–12).

The aim of the study presented here was to investigate the influence of arteriolar vasomotion on the cutaneous oxygen supply. Particular attention was paid to whether there were differences in variations of capillary haemoglobin saturation between various regions of the body. The intraindividual variation coefficients for the oxygen saturation of haemoglobin and the haemoglobin concentration range from 4.6–25.3% (10). As the absolute values, as with those in laser Doppler flowmetry, can be utilized only with reservations, in the following study only relative changes over time at a particular measuring site were examined.

MATERIALS AND METHODS

Reflection spectrophotometer

We used a prototype of a reflection spectrophotometer (MULTISCAN OS10, NIOS GmbH, Germany) (8). A 50W halogen lamp emits white light with a wavelength range of 400–1100 nm. The light is guided to the skin and back by 1000 50-μm fibres. The fibres are arranged randomly within a single cable with a diameter of 1.5 mm and a length of 1.6 m. The diameter of the light path is therefore large in relation to the capillary diameter of 10 μm, so that movement artefacts play a relatively small role.

The received light passes a scanning holographic grating and the resulting split spectral components are sampled one after the other by one detector (principle of monochromatism). The detected spectrum covers a range from 400–1070 nm. Eight spectra per second are sampled. The digitized signals are recorded, averaged every second and immediately evaluated by a microcomputer system.

Data analysis

The spectroscopic data are transformed to a log (1/R) scale, mean-centred and normalized. The law of Lambert-Beer is not suitable for the calculation of haemoglobin levels in tissue based on data obtained with reflection photometry, due to (i) inhomogeneous distribution of haemoglobin in tissue, (ii) other substances present in the tissue which contribute to the reflection spectra, and (iii) the unknown path length of the reflected light in the tissue. Thus, further data analyses were performed using software developed for this purpose (9).

The inhomogeneous distribution of the chromophore was determined as follows. If S (λ) denotes the measured reflection spectrum of the skin and E (λ) the absorption spectrum of haemoglobin solution in a cuvette, then a mapping M exists with: $E(\lambda) = M(S\lambda)$. M is a non-linear one-to-one mapping (9). This mapping M depends on the degree

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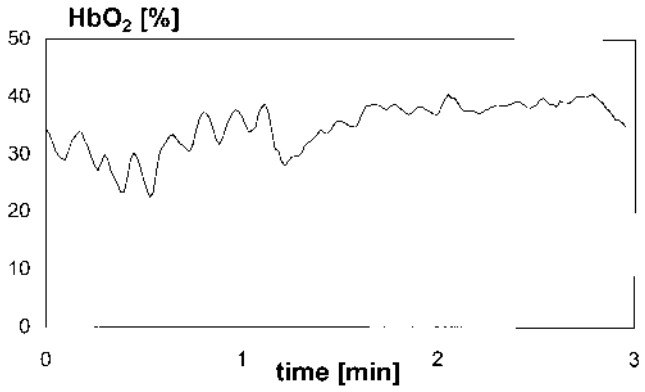

Fig. 1. Oxygen saturation of cutaneous capillary haemoglobin. Seven cycles of rhythmical variation per minute.

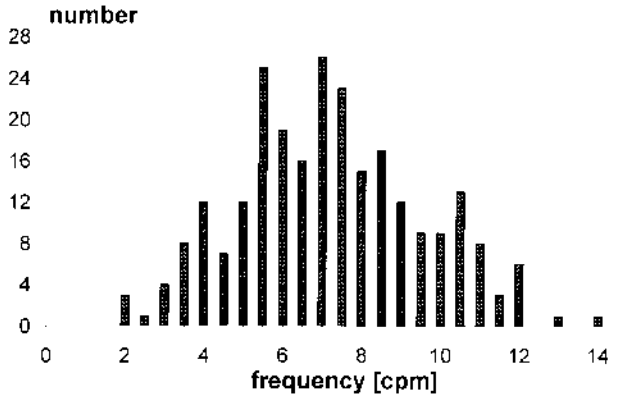

Fig. 2. Variations of haemoglobin oxygenation. Frequency prevalence in 265 spectra with rhythmical variations. Prevalence is given by the number of occurrences.

of inhomogeneity, not on the type of inhomogeneity (13), and is scale-invariant. Therefore, the degree of inhomogeneity can be calculated and an inhomogeneous spectrum can be corrected. The spectra obtained in this study were processed in this way.

It is necessary to correct for the influence of other components on the reflection spectra. Since the statistical methods are sensitive to changes in measuring conditions, it is possible to assign the deviation to the compound of interest (14). Applying partial component regression and a partial least square multivariate algorithm, the deviation due to the known haemoglobin spectrum can be assessed, if non-linear distortion of this spectrum has been previously corrected (15). Since an inverse mapping of M exists, it has been demonstrated that it is possible to alter M in such a way that the estimated square of the deviation approaches a minimum (16). The spectrum is normalized for a suitable concentration. For each normalized spectrum, multivariate analytical methods were applied (17).

The temporal course of oxygen saturation of haemoglobin ($HbSO_2$) (%), of the concentration of oxygenated haemoglobin (HbO_2) (mg/mL) and of the haemoglobin content (Hb) (mg/mL) were examined.

Subjects and experiments

In order to examine the frequency of rhythmical variations of the oxygen saturation depending on the locality, 20 healthy volunteers (10 women, 10 men, mean age 39.8 ± 13.9) were examined on 24 different sites of the body. Eight different sites were measured on the head (forehead, cheek, lip, nose, chin, lid, ear, neck), 4 on the trunk (back, seat, breast, abdomen), 6 on the upper extremities (upper arm, forearm, hand dorsal, hand palmar, finger dorsal, finger palmar) and 6 on the lower extremities (thigh, calf, foot dorsal, foot plantar, toe dorsal, toe plantar). Exclusion criteria were heart failure, lymphoedema, restricted mobility, skin diseases, diabetes mellitus, arterial hypertension, peripheral arterial occlusive disease, chronic venous insufficiency and lung diseases.

Use of nicotine was stopped 2 h before measurements. After a resting period of 20 min, the skin reflection spectra were measured for at least 150 s with the volunteer lying flat in a room with a temperature of 22°C (room temperature), in the afternoon. The measuring head was placed on a 1-mm thick metal clip with a 9-mm wide central opening, allowing light to pass through. Thus, direct skin contact of the actual measuring head could be avoided, which, in turn, allowed assessment of the measuring site without application of unwanted pressure. Skin contact, however, was allowed at a distance of roughly 4 mm away from the measuring site through the metal clip. This allowed minimization of movement artefacts. Using this technique, the airflow to the skin was not disrupted so that warming of the skin did not occur. In order to reduce artefacts from other pigments of the skin, all spectra measured in perfused skin were subtracted from one previously measured spectrum of haemoglobin-free skin of the examined subject. To accomplish this, the blood was pressed out of the dermal vessels by applying high pressure to the probe and against the bone of the medial epicondyle (10).

Analytical method for temporal variations

Periodical variations are usually analysed with Fourier transformation algorithms like Fast Fourier Transformation (FFT) or Digital Fourier Transformation (DFT) observing the power spectra. The time course of spectra examined in our study (sample frequency 1/s) was characterized by a relatively long duration of the periods as compared to the duration of the measurements (Fig. 1). We therefore used a new filter of the DFT, which allowed us to study these relatively long periods in relation to the relatively short measuring time. Longer measuring times were, due to the increasing movements of the volunteers, not practicable. This small box function in the time domain leads to a very strong center peak in the frequency spectrum. The frequency positions of the sidelobes of this strong peak are in the order of the vasomotion frequency. For assessing the vasomotion, this artificial frequency peak has to be suppressed. Two steps are important for this algorithm:

1. A convolution with a high-pass filter function suppresses the boxcar function and the low frequency variations. The filter function is time-symmetric. The low cut-off frequency can be varied by the brightness of the filter function.
2. A specially smoothed apodization function gives a better relation between peaks and their sidelobes in the frequency spectrum.

The time curves of $HbSO_2$, Hb content and HbO_2 were visually compared to detect synchronous variations.

RESULTS

In 265 out of 480 spectra (55.2%), periodic variations of the haemoglobin oxygenation were found. These periodic variations could also be demonstrated in the raw data. The average frequency of the analysed spectra was 7.0 ± 2.5 cycles/min (cpm) (Fig. 2). The occurrence of variations of the haemoglobin oxygenation depended on the measuring site. In the gluteal region, they were observed in 17 out of 20 subjects, on the palms in 16 out of 20, at the foot plantar in 18 of 20 in comparison to the cheek (8/20), the lip (5/20) and the eyelid (6/20). The haemoglobin oxygenation ($HbSO_2$ (%)) varies in synchrony with the changes of the capillary haemoglobin concentration (Hb content (mg/ml)). On the head, we observed significantly more variations per minute than in the lower extremities (Fig. 3) ($p \leq 0.0001$, Student's *t*-test for linked

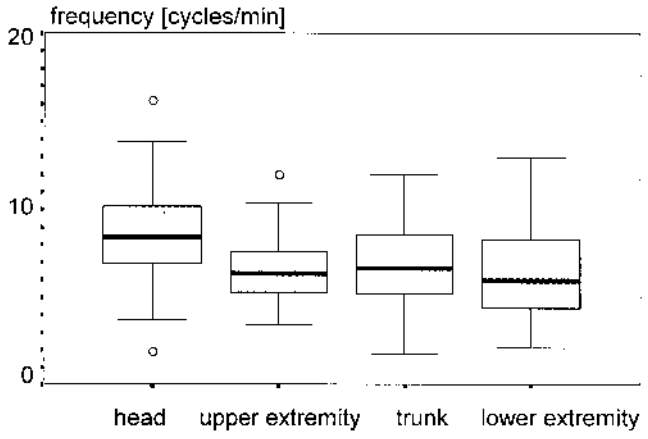

Fig. 3. Frequencies of variations of haemoglobin oxygenation. Note significantly higher frequencies at the head than at the lower extremity ($p \leq 0.0001$).

samples, SPSS for windows, SPSS, Germany). The median amplitude of the variations of saturation measured 20% (range 5 – 50%).

DISCUSSION

Previously, short-term changes of cutaneous perfusion with durations of seconds could only be assessed with the aid of laser Doppler flowmetry (18 – 26). While this technique measures the perfusion in the subpapillary plexus, it fails in assessing oxygen supply to the skin. The oxygen supply to the skin can be derived from the transcutaneous oxygen pressure ($tcpO_2$). For $tcpO_2$ measuring, however, heating of the skin is necessary to increase pO_2 on the cutaneous surface (27). At a skin temperature of 44°C, vasomotion is completely lost (27). As the measurement of the $tcpO_2$ quantifies relatively slow diffusion processes, it is not possible to examine processes that last only a few seconds. In our study, these problems have been solved by using a reflection spectrophotometer, which evaluates multiple spectra at physiological skin temperatures (9 – 12).

In the present study, we demonstrated rhythmical variations of the haemoglobin oxygenation in cutaneous capillaries with a frequency of 7.0 ± 2.5 cpm. They occurred with great regularity at rest and on the entire body. In one of the first studies on oxygen saturation of cutaneous haemoglobin, slightly slower frequencies of 3 – 5 cpm had been observed, but these frequencies were not systematically quantified by means of special software (10).

The periodic constriction and relaxation of the arterioles manifests itself in laser Doppler flowmetry as so-called "flow motion". Different working groups distinguish mostly between low-frequency waves of flow motion (average frequency 1 – 3 cpm) and high-frequency waves of flow motion (frequency in the range of 6 – 25 cpm) (19 – 24). In our investigations, we most frequently detected frequencies of 5.5 – 7.5 cpm. These frequencies correspond conspicuously with the rhythmical variations caused by vasomotion, as mentioned above. The frequencies measured by the reflection spectrophotometer are close to the high frequency waves in laser Doppler flow motion. The origin of these waves is not fully understood, but there is some evidence that the fast frequencies arise from the terminal arterioles. This supposition is supported by animal studies using hamster skinfold preparation. Here the frequency of vasomotion was lowest (3 cpm) in the large arterioles (diameter 70 – 100 μm), and increased successively to about 10 cpm in the terminal, precapillary arterioles (diameter 6 – 15 μm) (25). In human skin, over the ascending arterioles to the upper horizontal plexus (17 – 26 μm diameter), a flow motion with 6 – 10 cpm was found (24). These frequencies correspond exactly with those measured with the reflection spectrophotometer.

Our results and those mentioned above, prove that rhythmical oscillations in microcirculation subsist without provocation. During provocation, however, the amplitude of the waves increases markedly. In our data, periodic variations of the haemoglobin oxygenation could be found in 55.2% of the measurements. In laser Doppler flowmetry, rhythmical variations of the flow values could be observed in 80% of healthy subjects and 25% of patients suffering from arterial occlusive disease (23). The reason for the lack of vasomotion in some cases remains unclear. Perhaps it could be explained by the sensitivity of the measurement techniques, but it seems to be independent from the measuring time (own unpublished data). Since vasomotion is influenced by temperature, the higher temperature in the face together with the thinner skin is perhaps the explanation for the absence of the spectra in the face.

At present, the physiological function of rhythmical variations of the haemoglobin oxygenation of capillary blood and its importance for human skin remains unclear. As these variations display an amplitude of up to 50% and a relatively slow dynamic, with a duration of up to 30 s, in comparison to heart rate and frequency of breathing, consequences for cutaneous diffusion and metabolism of other substrates seem quite likely, and should therefore be considered in studies to come.

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