Evaluation of haemoglobin changes of skin and muscle tissue of the calf induced by topical application of a nonivamide / nicoboxil cream

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ABSTRACT
Topical agents inducing hyperaemia like nonivamide or nicoboxil increase cutaneous blood flow and temperature and induce erythema. It is not proven up to now whether there is also a hyperaemisation effect in skeletal muscle. This study has the objective to determine the effects of a nonivamide / nicoboxil cream on haemodynamics in skin and calf muscle via optical spectroscopy in the visible and near-infrared with a separation of changes for skin and muscle. Left and right calves of 14 healthy subjects were treated with a nonivamide / nicoboxil cream or mock administration, and cutaneous and muscle haemoglobin were measured using a combined NIRS / VIS sensor. The topical application of the cream increased the concentration of oxygenated haemoglobin and tissue oxygen saturation significantly in skin as well as in muscle of the treated legs already after 15 minutes, with stronger and faster effects in skin. In contrast, the change in deoxygenated haemoglobin was found to be small. The kinetic of all changes varied widely between the subjects. The found haemoglobin changes might explain the beneficial effect of hyperaemia creams for the treatment of minor injuries.

Keywords: Optical spectroscopy, Near Infrared spectroscopy (NIRS), hyperaemia, creams / ointments, skin and muscle oxygenation, haemoglobin, nonivamide, nicoboxil

1. INTRODUCTION
The topical application of hyperaemia inducing creams and ointments features a long history within treatment of various musculoskeletal injuries and disorders. Products which contain capsaicin or related agents like the synthetic capsaicin-analogue nonivamide (N-Vanillyl-Nonamid), esters of nicotinic acid like nicoboxil, or a combination of both are examples for such topical preparations. They are not only popular for the afore-mentioned treatment of various disorders of the musculo-skeletal system, but also to enhance the working conditions of human muscle before initiation of physical activity. Such a combination product ("Finalgon®") was developed in the 1950s in Germany and still is very popular in several European countries.

Scientific studies which describe the hyperaemic effects, as well as clinical efficiency, are rare and were mostly published in the 1950s [1 - 13]. Back then, though, the available technology limited the researchers in a detailed description and quantification of the hyperaemic effect.

In many studies skin temperature was used as an indicator for subcutaneous blood flow [8, 10 - 14]. However, tissue temperature can be influenced by several physiologically parameters even at constant room temperature and is therefore not reliable for the estimation of skin blood flow [6]. Evidence was found for the effects of the nonivamide / nicoboxil cream on skin blood flow in 1996 via laser-Doppler-scanning in collaboration with colorimetry and skin temperature measurement [14]. Early radioactive tracer experiments indicated the increase of muscular blood flow through application of the nonivamide / nicoboxil combination [7, 8, 10, 15]. This effect could explain the beneficial effects on muscular disorders, though the findings were limited to a relatively short duration of 20 minutes after application. These data have never been confirmed by other methods and the full kinetics of the hyperaemic effect could not be quantified.
Furthermore, the depth sensitivity restricted an analysis either to superficial tissues or did not allow a discrimination of different tissues. Hence it can be subsumed that up to today no complete statement can be made about the influence of a nonivamide / nicoboxil cream on skin and muscle blood flow. Furthermore, no research has been undertaken to show the effects on other haemodynamic parameters like haemoglobin concentration and oxygen saturation which are linked to an increased tissue blood flow.

Near Infrared Spectroscopy (NIRS) is a suitable method to assess an increased muscular blood flow by measurement of haemoglobin changes in skeletal muscle. Alongside with the wide use of NIRS in clinical studies [16] this type of diagnostic is becoming more significant for sport and exercise science in the area of the encephalon and muscle skeletal research [17, 18, 19]. Nioka et al. [20] pointed out that NIRS can provide good information as a method of non-invasive monitoring of blood flow. Furthermore the oxygenation of muscle is precisely determinable through this method [19]. The objective of this study is to describe effects of a cutaneous administrated nonivamide / nicoboxil cream on haemodynamic parameters in the treated skin by visible wavelength spectroscopy as well as the underlying muscle by NIRS.

2. METHODS

Subjects

14 male subjects without peripheral vascular diseases participated in this study. Their average age ± standard deviation (SD) was 26 ± 4 years. The adipose tissue thickness (ATT) was determined prior to optical monitoring by ultrasound imaging (Sonoline G40, Siemens, Germany) to exclude its impact on NIRS. According to previous publications the ATT should not exceed 15 mm [21, 22]. The assessed ATT of our subjects in the region cruris posterior, right above the M. soleus and M. gastrocnemius caput laterale, was 6.2 ± 1.4 mm.

The study was approved by the local ethics committee of the German Sport University and was in line with the Declaration of Helsinki. Every subject was informed in writing and verbally about any possible risks of this study. In addition they had to sign a declaration for exclusion of liability.

![Figure 1](http://proceedings.spiedigitallibrary.org/)

Figure 1 a) Attenuation spectra for VIS and NIR sensors, each for two different source-detector distances. Measurements were taken at the start directly after application of Finalgon (dashed line) and at the time of maximal changes (solid line). The attenuation for VIS and NIR is arbitrarily scaled. b) Haemoglobin extinction spectra. For the NIR range the spectra are scaled by a factor of 20.
**Optical spectroscopy set-up**

For the monitoring of haemoglobin two fibre based optical spectroscopy systems were used with different depth sensitivity profiles for a discrimination of superficial tissue (‘skin’) and deeper layers (‘muscle’). The first system is based on the visible wavelength range (VIS) from 480 – 650 nm where the absorption of light is high and dominated by haemoglobin. This high absorption combined with a small separation between light source and detector fibres limits the photon penetration depth and therefore the probed tissue depth. The hardware prototype is the basis of a commercial product (moorVMS-OXY, Moor Instruments Ltd., UK) based on the same principle and algorithm [23]. The light delivering optical fibre (diameter: 0.2 mm) and the detector fibre (diameter: 0.6 mm) were positioned at six distances of 1.9 to 3.3 mm with direct contact to the skin. Light attenuation spectra were averaged for these fibres and analysed by fitting the extinction spectra of oxygenated and deoxygenated haemoglobin (oxyHb and deoxyHb, respectively) and melanin. Subsequently, the oxygen saturation of tissue was calculated as \( SO_2 = \frac{\text{oxyHb}}{\text{totHb}} \).

The second system detected reflectance spectra for source-detector distances \( \rho = 23.5, 26.5 \) and 30.0 mm in the near-infrared (NIR) range from 720 – 860 nm where the absorption of haemoglobin is lower by up to two orders of magnitude compared to the VIS range. An earlier version of the system and the underlying method is described in the literature [24, 25, 26]. Two approaches were followed for the NIR data analysis. First, the modified Lambert-Beer (MLB) law [27] was applied which states that changes in light attenuation \( \Delta A(\lambda) \) at a wavelength \( \lambda \) are the product of changes in the absorption coefficient \( \Delta \mu_a \) of tissue, the source-detector distance \( \rho \) and the differential path-length factor DPF which corrects for the longer mean path-length of photons in tissue [28]. From the broad-band spectra of attenuation, changes in haemoglobin concentrations were calculated by matrix inversion. Second, the spatially-resolved spectroscopy (SRS) approach based on the derivative \( \partial A/\partial \rho \) of attenuation \( A \) with respect to the source-detector distance \( \rho \) was used to calculate absolute absorption coefficients \( \mu_a \) [29, 30, 24].

![Figure 2 Time course of haemoglobin changes for a representative subject over approximately 95 min after administration of a nonivamide / nicoboxil cream (t = 0 min). a) VIS data for skin (oxyHb, deoxyHb, SO2), b) NIR (SRS) data of absolute values in totHb and SO2 and c) NIR (MLB) data for changes \( \Delta \text{oxyHb} \) and \( \Delta \text{deoxyHb} \).](image-url)
Again, matrix inversion allows the absolute concentrations of haemoglobin to be calculated. As the SRS-based haemoglobin parameters are absolute numbers, the oxygen saturation \(\text{SO}_2\) can be obtained. In contrast, the MLB-method gives changes in concentration only and therefore no saturation values.

**Experimental design**

The optical fibres of the NIR system were positioned and held in a line on the skin by a plastic holder with the fibres of the VIS system positioned about 15 mm from the NIR detector fibre of largest \(\rho\). An additional plastic ring holder was fastened on the skin by bandage to fixate the position. Four of these ring holders allowed to take the fibre probes on and off for sequential recording on two sites of two subjects. A commercially available cream (Finalgon® Creme, Boehringer Ingelheim GmbH & Co. KG, Germany) containing 0.17 and 1.08% (w/w) of nonivamide / nicoboxil, respectively, was topically applied on the skin (approximately at the region cruris posterior right above the soleus muscle and gastrocnemius muscle caput laterale) at a dose of appr. 250 mm³ (about 1 cm of cream) for an area of 90 mm x 60 mm. For the long time recording of haemoglobin changes the optical sensor were directly attached after administration of the cream and optical spectra recorded every 2 s. For the comparison between tissue treated with the cream and reference tissue, the cream was administered to the skin of the left calf of the subjects and rubbed for 10 s. To test for possible artefacts due to the manual stimulation of tissue blood flow, the right calf was manipulated at the corresponding area mimicking cream administration. Haemoglobin was monitored on both sites in sequence. To avoid the spread of the cream between the tissues by the replacement of the optical probe, a thin transparent foil was placed between skin and probe. Since the foil acted as an optical waveguide and increased the light intensity transported to larger distances from the light source, only the MLB-analysis was applied in this case.

The main objective here, the discrimination of haemoglobin changes in different tissue layers, requires an estimate of the depth sensitivity which can be derived from mathematical models for the light propagation in scattering media. For VIS wavelengths and \(\rho = 1.9\) to 3 mm used here, the likely mean depth sensitivity is about 1 – 2 mm, and therefore the signal can be attributed to ‘skin’ with no contribution from ‘muscle’ tissues. For the NIR data the depth sensitivity depends not only on the source-detector distance and the optical parameters of the tissue but also on the analysis. Estimates from Monte Carlo simulations [26] suggest for the MLB-method that the mean depth of sensitivity is about 8 - 12 mm while it is about 14 - 16 mm with reduced contributions from the upper tissue for the SRS-analysis. Therefore, the SRS-based data of haemoglobin can be attributed to ‘muscle’ with only minor contributions from upper layers like skin or adipose tissue. For MLB the contribution of adipose tissue is somewhat larger. In what follows it is assumed that the NIRS data contain signal from ‘muscle’ only when strictly speaking there is a minor contribution from the upper tissue layers. This is especially justified as the subcutaneous adipose tissue contains less haemoglobin.

Data was gained for three minutes of each calf every 15 min up to 90 - 93 min. Thus it was possible to obtain data from both calves of two subjects in the same measurement cycle through transfer of the optical sensors. The application of the cream occurred 30 s prior to the first measurement of the corresponding calf with duration of ten seconds. The mock-treated calves received a ten second placebo inunction without any substance 30 s prior to their first measurement as well. The impact of the inunction on the blood flow could therefore be excluded. For each calf data were eventually collected at times 0 – 3 min, 15 – 18 min, 30 – 33 min, 45 – 48 min, 60 – 63 min, 75 – 78 min and 90 – 93 min. For each interval data of the last two minutes were averaged to exclude the impact on haemodynamics by the replacement of the sensor.

Instrument control and data acquisition was programmed in Labview (National Instruments Inc., USA). All post processing was done in Matlab (The Mathworks Inc., USA). An ANOVA and a Duncan test were performed in Statistica 8 (StatSoft Inc., USA). The significance level was set to \(p \leq 0.05\).

**3. RESULTS**

Typical attenuation spectra are shown in Fig. 1a, both for the VIS (source-detector distance \(\rho = 2\) and 3 mm) and NIR (\(\rho = 23.5\) and 26.5 mm) range. The attenuation scale is fixed for the VIS and NIR spectra, however arbitrarily set between VIS and NIR. The variation in the shape from the initial spectra (dashed line) to those after hyperaemisation (solid line) can be understood by comparison with the extinction spectra of haemoglobin. E.g. the strong double-peaked extinction spectrum of the oxyHb extinction in the VIS appears more prominent in the attenuation spectra after the hyperaemisation effect. Based on these spectra the haemoglobin parameters were calculated.

The time course of the haemoglobin parameters is shown for a typical subject in Fig. 2. As was to be expected after the administration of a hyperaemisation-inducing agent, application of the nonivamide / nicoboxil cream (\(t = 0\) min) induced...
an increase in the cutaneous concentration $\Delta$oxyHb with a concurrent but smaller decrease in $\Delta$deoxyHb (Fig. 2a). This is reflected in a rise in SO$_2$ from about 40% to 80%. All of these changes in the skin parameters remain remarkably stable after the first 15 min. In the muscle below the cream treated area, total haemoglobin (totHb = deoxyHb + oxyHb) increased slightly while SO$_2$ remained more or less constant (Fig. 2b, SRS - analysis). When focusing on the MLB-data, there is an increase in oxygenated haemoglobin (AoxyHb) with minor changes in $\Delta$deoxyHb (Fig. 2c). It is apparent that the kinetics was quite different from the skin data with the muscle values showing a slow, steady increase in AoxyHb for about 80 min. Differences were found between the SRS- and MLB-data which might be due instrumental limitations or differences in the depth sensitivity.

Figure 3 Time course of oxyHb changes for the initial phase after application of a nonivamide / nicoboxil cream (t = 0 min). Each subplot corresponds to one subject with the VIS ('skin') and NIR ('muscle') data in thick and thin lines, respectively.

Similar measurements were performed in 9 subjects, which revealed an increase in oxygenation in all cases with a wide distribution both in onset times and kinetics. The variety of kinetics is shown for three data sets in Fig. 3 where the changes in oxygenated haemoglobin AoxyHb illustrate a high inter-subject variability and large differences in the relative kinetics of skin and muscle. These differences obstruct a simple analysis of the kinetics: the onset of the $\Delta$oxyHb changes spreads between about 1 min (Fig. 3c) and up to 5 min. Similarly, the time to half maximum ($T_{50\%}$) shows a wide distribution from about 1 min to 20 min for skin and about 4 to 40 min in muscle. Besides that, the three examples demonstrate a kinetic with similar (Fig. 3a) or slower (Fig. 3b) rise times of muscle compared to skin. However, in all subjects the administration of the nonivamide / nicoboxil cream augmented cutaneous, as well as muscular AoxyHb.

While these data describe the differences in the kinetics between skin and muscle data, the following data (Fig. 4) depict differences between untreated tissue (reference, R) and tissue treated with the nonivamide / nicoboxil cream (in the figure marked by R and N/N, respectively).

The time lag between R- and N/N- data is due to the positioning of the sensor probe on the two measurement sites (left and right calf). OxyHb of skin (Fig. 4a) increases more than twofold with the cream ($p \leq 0.001$) and shows a slight increase only in the reference tissue, probably due to the mock application of the cream. By comparison, deoxyHb changes are small. Similarly for muscle (Fig. 4c), AoxyHb is much larger at the cream treated site than at the reference site ($p \leq 0.001$). AdeoxyHb hardly changes from baseline values. These findings hold for all measurement points starting from 15 – 18 min after administration of the cream. The effect on AoxyHb in both tissues of the treated leg was observed in all subjects and is therefore significant even up to the last measurement point at 90 - 93 min in comparison with the reference leg. The maximum of the effect in skin is recognizable in the fourth measurement point (45 - 48min), whereas in muscle it was found within the fifth measurement point (60 - 63min). SO$_2$ of skin (Fig. 4b) increases from about 60 to about 75 % after 15 minutes, with a much less pronounced increase in the reference tissue. This effect remained up to the last measurement point at 90 –93 min. Altogether oxyHb and SO$_2$ of skin as well as AoxyHb of muscle increase in all subjects. Though the limited number of data points prevents a detailed analysis of the kinetics, the fast rise of the skin values compared to muscle data is apparent.
Figure 4 Haemoglobin parameters as mean (± SD) for 14 subjects for nonivamide / nicoboxil cream treated tissue (N/N, solid symbol) and reference tissue (R, open symbol) is shown. In a) and b) VIS (‘skin’) concentration changes Δc of oxyHb and deoxyHb as well as SO2 are shown while c) depicts NIR (‘muscle’) data of changes ΔoxyHb and ΔdeoxyHb. The cream was administered at t = 0 min.

4. DISCUSSION

Hyperaemisation-inducing topical treatments for soothing muscular-skeletal complaints are very common. The effects on haemoglobin in skin and muscle, however, have not been analysed in detail. This study demonstrates cutaneous and subcutaneous changes of haemoglobin after the administration of a nonivamide / nicoboxil cream. An increase of oxyHb in skin and muscle was observed in all subjects, with an onset commencing as soon as 1 min after administration of the nonivamide / nicoboxil cream. The onset and the kinetics of oxyHb in skin and skeletal muscle after the nonivamide / nicoboxil treatment were found to be different. Furthermore the presented data give evidence how fast the active ingredients of the cream induce local effects on the blood perfusion and oxygen supply into subcutaneous regions.

Our results for the skin tissue are in line with findings of previous studies where skin blood flow was examined via laser-Doppler-scanning [14]. Stücker et al. [31] described the effects on cutaneous blood circulation in detail for nonivamide, nicoboxil and the combination of both compounds. In addition to these previous findings, our investigation demonstrates that administration of the nonivamide / nicoboxil combination augments cutaneous concentration of oxyHb and elevates SO2. More important for medical and sport practice is the precise finding of a ΔoxyHb increase in muscle tissue after topical application of a nonivamide / nicoboxil cream. The data of our study is descriptive and points at a vasodilatation in muscle tissue as the total haemoglobin concentration increases. However, the data does not allow the mechanisms of action in muscle to be inferred. Similarly, most previous studies were not able to address this problem either. The main mechanism for the tissue perfusion changes is believed to be a reflective effect from skin to muscle tissue [11, 32].
data, though, indicates that a diffusion of the substances might contribute. The determined effect of the nonivamide/nicoboxil cream on haemoglobin and its oxygenation was significantly higher in skin in comparison to muscle (p ≤ 0.001) which could indicate that not the whole amount of the topical administered substances is able to diffuse into the deeper tissue. This could explain the more pronounced effect in skin. Our method was able to determine effects up to approximately a 25 mm tissue depth and a depth of highest sensitivity of about 10 – 12 mm, which is twice as much of measured ATT. Measurements within even deeper regions in bigger muscle groups (e.g. M. quadriceps femoris) might show a further decrease of the cream’s effect on tissue perfusion. Furthermore, the determined ΔoxyHb maximum was found to appear earlier in skin (fourth measurement point within 45 – 48 min) in comparison to muscle (fifth measurement point within 60 - 63min). Therefore it can be speculated that the delay of maximal muscle response is explained by the larger diffusion distance. Further investigations, however, are needed to clarify the issue whether diffusion of the cream’s active ingredients, or other mechanisms explain the haemoglobin concentration changes in skin and muscle.

The NIRS signal originates mainly from erythrocyte Hb in arterioles, capillaries and venules [33]. The area of capillaries is known as the main location for metabolite exchange with deoxygenation of haemoglobin [34, 35]. Our study revealed that the topical application of a nonivamide/nicoboxil cream increases ΔoxyHb concentration in muscle tissue with minor changes in deoxyHb which can be derived from higher concentration or a reduced deoxygenation of haemoglobin in blood vessel of the muscle [18]. An opening of arterioles, capillaries and venules of skeletal muscle increases blood volume and the blood flow in skeletal muscle [36]. An increased blood perfusion within the muscle creates many known advantages for physical activities like improved metabolite exchange or a lower prevalence and risk for muscle injuries. Here we found that the ΔoxyHb concentration in muscle tissue is notably higher than the ΔdeoxyHb concentration after topical application of a nonivamide/nicoboxil cream. This indicates an increase of SO2 in muscle which improves conditions for physical activity as well. Therefore this might indicate that the nonivamide/nicoboxil cream can not only be used for injury prevention but also for an improvement of metabolic conditions prior to physical activity. Nonivamide/nicoboxil cream may also be used for treatment of many minor muscle injuries like a delayed-onset muscle soreness as blood flow increasing actions are well known to promote tissue regeneration through an improved nourishment [37]. Likewise many further minor muscle injuries can be treated by blood flow increasing actions within the affected body area through improved tissue nourishment [37].

5. CONCLUSION AND PERSPECTIVES

Our data shows that VIS/NIR spectroscopy is a reliable method to simultaneously monitor blood oxygenation parameters in skin, as well as in muscle. Furthermore it supports many statements made in the 1950s years that a nonivamide/nicoboxil cream can be used to treat various kinds of minor muscle injuries through promoted tissue regeneration. In addition it may be used prior to physical activity for local injury prevention or metabolic improvement. Further studies may focus on the effect of the creams in deeper muscle regions and on the precise effect on muscle oxygenation. Similar importance lies in investigations of the mechanisms behind the effect of the cream in muscle tissue and in studies which focus on precise treatment effects of the cream on minor muscle injuries.

Competing interests: The laboratory at RheinAhrCampus received funding by the producer of Finalgon, Boehringer Ingelheim GmbH & Co KG, for the development of optical methods for the assessment of tissue haemoglobin.

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