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Muscle capillary behaviors during a fatiguing isometric action
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Behavior of oxygen saturation and blood filling in the venous capillary system of the biceps brachii muscle during a fatiguing isometric action
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Abstract
The objective of the study was to develop a better understanding of the capillary circulation in contracting muscles. Ten subjects were measured during a submaximal fatiguing isometric muscle action by use of the O2C spectrophotometer. In all measurements the capillary oxygen saturation of hemoglobin (SvO₂) decreased immediately after the start of loading and leveled off to a steady state. However, two different patterns (type I and type II) emerged. They differed in the extent of deoxygenation (0.37 ± 2.59 percent points (pp) vs 83.86 ± 17.35 pp, p = .008) and the behavior of the relative hemoglobin amount (rHb). Type I led to a positive rank correlation of SvO₂ and rHb (ρ = 0.735, p < .001), whereas a negative rank correlation (p = -0.522, p < .001) occurred in type II, since rHb decreased until a reversal point, then increased averagely 13% above the baseline value and leveled off into a steady state. The results reveal that a homeostasis of oxygen delivery and consumption during isometric muscle actions is possible. A rough distinction in two types of regulation is suggested.

Key Words: muscle oxygenation, hemoglobin amount, isometric muscle action, O2C spectrophotometer

Isometric muscle actions play an essential role in daily activities which include posture or static work. During that type of muscular activity the intramuscular pressure increases proportionally to the maximal voluntary isometric contraction (MVIC). According to some authors the resulted mechanical pressure might impede the blood flow within the muscle, which is essential for an adequate oxygen supply. The blood flow could already be restricted at intensities of 15% MVIC. The comparison of studies which examine the relationship between muscle tension and blood flow is difficult because of an extensive range of methodologies and measurement techniques. Thus, it is still unclear which relative or absolute contraction intensity possibly causes a restriction or even a complete occlusion of the capillary vessels. But if the inflow is restricted or occluded the available oxygen ought to be depleted over time. As a result, the saturation might decrease to zero (complete deoxygenation).
Several studies examined the oxygen saturation of different muscles during sustained isometric contractions with various intensities and exercise durations. Most of them used the near infrared spectroscopy technique (NIRS), which primarily reflect the oxygen saturation of small veins (<1 mm diameter). The principle and other limitations are described elsewhere. Disregarding methodological inconsistencies, two different tendencies are described or shown graphically. On the one hand, the muscle oxygenation decreased and leveled off into a steady state after a certain time. This implies that the saturation stays nearly constant over time. On the other hand, it decreased discontinuously or in a phasic way with different decay rates until termination of the exercise.
According to Sadamoto and colleagues (1983), stopped outflow during isometric contractions due to a possible venous stasis should be considered, too. This be verified by measuring the hemoglobin concentration as an indicator of the blood flow. In most cases, the total hemoglobin amount measured by the NIRS method decreased and behaved like the oxygen saturation. However, there are also research groups which found an increase after an initial decrease, or a direct increase after the start of load. Inter- and intra-individual differences were also found.
Because of the inconsistencies mentioned above, the results should be verified by use of the diagnostic device O2C (Oxygen To See LEA Medizintechnik GmbH, Gießen, Germany). In contrast to the NIRS technique, O2C is able to detect only very small vessels (<100 µm diameter). Thus, measurements mainly represent capillary-venous oxygen saturation of hemoglobin (SvO₂) and the relative hemoglobin amount (rHb) per local tissue volume. The device is usually used for non-invasive determinations of oxygen supply in microcirculation of blood perfused tissues and commonly applied in surgery like organ or flap transplantations, monitoring processes of diabetic diseases, or during arterial occlusive disease.

This study questions how SvO₂ and rHb behave in muscle tissue during a fatiguing isometric action despite a potentially stopped blood flow.

Materials and Methods

Subjects

The left arm of seven male and three female healthy Caucasian volunteers (mean age ±SD = 28.6 ±11.68 years) were examined. They included students, colleagues of the University of Potsdam and local acquaintances. The exclusion criteria were any kind of chronic or acute health problem. The participants weighted 70.2 ±11.8 kg on average and were 176.3 ±8.6 cm tall. Based on the BMI (22.44 ±2.19 kg/m²) everyone was normal weighted. The study was conducted according to the declaration of Helsinki and local ethical permission was given. All subjects were informed in detail and gave their informed written consent to participate.

Measuring technique

The noninvasive O2C device was utilized to record SvO₂ in % and rHb in arbitrary units (AU). A calibration with another method measuring e.g. milliliter per gram of tissue is missing. However, for this study the curveshape is more important than a quantitative comparison. The sampling rate was 40 Hz. Preliminary studies revealed that the device is valid, reliable and also applicable to muscle tissue at rest or during exercise. The principle of the measurement relies on a combination of laser Doppler technique and tissue spectrophotometry (laser light: near infrared, continuous wave, power >30 mW; white light: 500–850 nm, 1 nm resolution). In this study, only the spectrometry for a detection of SvO₂ and rHb is relevant, whereby white light is sent into the tissue and the detection of different backscattered wavelengths of oxygenated and deoxygenated hemoglobin is used for their calculation. A detailed description of the techniques can be found in previous studies.

During all measurements the room light was dimmed to minimize light effects on the probe. We monitored the parameters in muscle with a depth up to 12 mm. For this purpose, the measuring probe (LF3, separation: 16 mm) was directly stuck on the skin over the anterior side of the belly of the biceps brachii muscle and along its fibers by use of a doubled adhesive film.

Setting and procedure

At the beginning the MVIC of each subject was determined. Six subjects pulled a weight maximally on a fixed strain gauge (resting period: 2.53 ±0.33 min). These two MVIC-tests were recorded by the O2C device (sub group analysis). The other six subjects should hold the highest possible weight for 1 s within maximal 5 steps. In both versions the arm position was identical to the subsequent fatiguing trial. For this, everyone had to hold a weight of 60% of the MVIC until fatigue. This intensity was chosen to generate a high intramuscular pressure in order to ensure theoretically a nearly full occlusion of capillaries. Furthermore, a loading duration which might be long enough for a maximal deoxygenation and short enough to minimize an early stop because of reasons of motivation.

Figure 1 illustrates the measurement position. The subject stood upright habitually. The upper arm was adducted, the forearm was supinated and positioned horizontally (90° elbow flexion). A cuff was applied 2–3 cm proximal to the wrist crease. The rater hooked the respective weight onto the cuff (here in after referred to as loading). Subjects were instructed to maintain the elbow angle for as long as possible. The weight was taken off as soon as the angle of the elbow exceeded 90° for more than two seconds assessed by the rater subjectively. The measuring record started 10 s before loading and was stopped after two minutes of recovery.

![Fig. 1 Measurement position and set up.](image)
Data processing and statistical analysis

The graphs of the raw data of the fatiguing trials and MVIC-tests were initially viewed in NI DIAdem\textsuperscript{TM} 2012. For further calculations all curves were smoothed (moving average, maximal smoothing width: 50). To describe and cluster the patterns of behavior of the parameters ($SvO_2$, rHb) the following variables were analyzed:

i. coefficient of variation (CVs) of a possible steady state

ii. the slopes from start of loading to leveling off into a possible steady state

iii. the durations from start to the leveling off and to the end of the possible steady state

iv. extents of alteration in relation to baseline values

Firstly, two intervals were defined in each curve. The boundaries of the first interval (I\textsubscript{1}) were set from start of loading to the following first local minimum ($1^{st}$ Min.), Figure 2A, left panel. The boundaries of the second interval (I\textsubscript{2}) were set in two different versions depending on curve progression. If the $1^{st}$ Min. is equivalent to a reversal point (RP), i.e. a direct continuous increase follows the first interval, the start of I\textsubscript{2} was set at the first local maximum ($1^{st}$ Max.) after the continuous increase (Figure 2B, right panel). If a RP does not exist the latter boundary of I\textsubscript{1} ($1^{st}$ Min.) corresponds to the start of I\textsubscript{2} (Figure 2A, left panel). In both versions I\textsubscript{2} ends at stop of loading.

To analyze, arithmetic mean (M) and standard deviation (SD) of all data points within I\textsubscript{2} were calculated. Subsequently the CVs within one subject were recomputed ($\frac{SD}{M} \times 100$). Furthermore, peak-to-peak amplitudes of the parameters within I\textsubscript{2} and their calculated means and SDs were extracted. Variable ii was quantified by least square regression line within the I\textsubscript{1} ($r_1$) and additionally, between I\textsubscript{1} and I\textsubscript{2} if a RP exists ($r_2$). However, regression lines were calculated under exclusion of the leveling off phase. For this purpose only data points according to the corresponding plateau of the first derivative were used for regression analysis. For iii, M and SD are stated in seconds (s).

Concerning iv baseline values were determined by averaging the initial 400 data points (10 s) in the unloaded measuring position. Alteration of parameters (extent of $SvO_2$ decrease (deoxygenation), extent of rHb decrease and increase, respectively) were calculated by calculating the differences from baseline values to the respective means of I\textsubscript{2}. They are presented in percentage points (pp) for $SvO_2$, AU for rHb and additionally in % for both.

The statistical analysis was made by use of IBM SPSS Statistics, Version 22. All variables were tested for normal distribution by the Shapiro-Wilk-test. Since the data were not normally distributed analysis of differences in curve patterns concerning the extent of deoxygenation were made by the exact Mann-Whitney-U-test. Confidence Intervals were estimated with the bias-corrected accelerated method (BCa 95% CI). Effect sizes were expressed by Cramer’s phi ($\phi$). Correlations of the parameters of each curve pattern were determined by Spearman’s rank correlation coefficients.

Results

The mean MVIC of all subjects was 279 $\pm$ 68.57 N.
Based on curve shapes, the behaviors of $S\text{vO}_2$ and $rHb$ during isometric actions could be differentiated into two patterns termed type I and type II. Figure 2A and B illustrate the different types using typical examples. They differ in the curve shape of $rHb$ and in the extent of deoxygenation. Five subjects were assigned to each type. All 3 female subjects belonged to type I. The BMI in type I was $21.89 \pm 2.54$ kg/m² and $23.00 \pm 1.85$ kg/m² in type II.

**Type I:** behavior of oxygen saturation and blood filling during fatiguing trials

$S\text{vO}_2$ and $rHb$ behaved nearly parallel to each other as illustrated in Figure 2A. At the start of loading both parameters decreased immediately and leveled off after averagely $15.1 \pm 1.6$ s. After the onset of recovery (after $49.72 \pm 12.35$ s on average) both parameters approached to or increased above the baseline value, respectively. The average CV of $I_2$ within subjects was $1.19 \pm 0.75\%$ in $S\text{vO}_2$ and $1.89 \pm 0.72\%$ in $rHb$. The peak amplitude of $I_2$ within subjects amounted averagely $2.45 \pm 1.37$ pp (min-max.: $1.364.74$) in $S\text{vO}_2$ and $3.39 \pm 1.14$ AU (min-max.: $1.724.75$) in $rHb$. The slope of $I_2$ amounted averagely $r = -1.69 \pm 0.92$ for $S\text{vO}_2$ and $n = -1.83 \pm 0.47$ for $rHb$. $S\text{vO}_2$ decreased by an average of $10.37 \pm 2.59$ pp ($= -13.38 \pm 2.75\%$) and $rHb$ decreased by averagely $-11.17 \pm 6.3$ AU ($= -18.22 \pm 9.03\%$) below its baseline value.

**Type II:** behavior of oxygen saturation and blood filling during a fatiguing trial

$S\text{vO}_2$ and $rHb$ showed a partial opposing behavior to each other as illustrated in Figure 2B. $S\text{vO}_2$ decreased immediately with an average slope of $r = -3.31 \pm 1.26$ and leveled off after averagely $15.55 \pm 3.23$ s. At the onset of recovery (after $41.89 \pm 14.10$ s on average) it approached to or increased above its baseline value. On the contrary, $rHb$ decreased immediately to a reversal point (RP) with an average slope of $r = -4.81 \pm 1.55$ on average and leveled off at nearly the same time as $S\text{vO}_2$ did. During recovery $rHb$ again decreased immediately to a second reversal point (RP 2) before it increased to its baseline value or higher. The average CV of $I_2$ within one subject was $2.11 \pm 1.59\%$ in $S\text{vO}_2$ and $1.41 \pm 0.75\%$ in $rHb$. The peak amplitude of $I_2$ within subjects $S\text{vO}_2$ and $rHb$ amounted averagely $2.67 \pm 1.77$ pp (min-max.: $0.93-5.15$) and $3.58 \pm 0.80$ AU (min-max.: $1.755.44$), respectively. $S\text{vO}_2$ decreased averagely $30.86 \pm 17.35$ pp ($= -41.46 \pm 22.4\%$). The $rHb$ increased by $9.03 \pm 10.48$ AU ($= 13.28 \pm 15.66\%$) on average over its baseline value.

**Behavior of oxygen saturation and blood filling during MVIC-tests**

During the 12 recorded MVIC-tests out of a subgroup of six subjects $S\text{vO}_2$ generally decreased followed by an immediate or a little delayed increase after stop of the test to the baseline value or higher, respectively. Nevertheless, the two patterns of behavior also occurred and were consistent within each subject. Figure 3A and B show typical examples. Except for one subject, everyone was grouped in the same type as referred to the fatiguing trials.

**Comparison of type I and type II**

The curve shapes of $S\text{vO}_2$ of both type were similar to each other. However, 1. the extent of deoxygenation and 2. the behavior of $rHb$ were different.

Concerning 1. arithmetic means and SDs of the extent of deoxygenation during loading of 60% of the MVIC-
Muscle capillary behaviors during a fatiguing isometric action

Eur J Transl Myol 30 (1): xx1-xx9, 2020

shown in Figure 4 A and during the MVIC tests in Figure 4 B with stated BCa 95% CIs. The rank sums of extents of deoxygenation differed significantly between the types in the fatiguing trials (U =2.61, p = .008, \( \varphi = .37 \)) as well as in the MVIC-tests (U =−2.72, p = 0.004, \( \varphi = .79 \)).

Concerning 2, type I had a positive rank correlation of \( \text{SvO}_2 \) and rHb from start until top of loading with \( \rho = 0.725, p < .001 \) on average. In contrast, \( \text{SvO}_2 \) and rHb in type II were negative correlated (\( \rho = -0.522, p < .001 \)). To illustrate the different types Figure 5 shows the differences between rHb and \( \text{SvO}_2 \) of all measurements.

Discussion

The authors suggest a distinction of two behavioral patterns (type I and type II) of oxygen saturation and blood filling of the venous microvessels during fatiguing isometric muscle actions. The crucial difference is the nearly parallel behavior of \( \text{SvO}_2 \) and rHb in type I expressed by a positive rank correlation and in contrast, the partial opposing behavior in type II with a reversed rank correlation.

A steady state of the considered parameters is characterized as an equilibrium of demand and supply with natural fluctuations. The presented within subject CVs of \( \text{SvO}_2 \) (0.31–4.33%) and rHb (0.5–2.88%) during the defined I2 seem to be low enough for a characterization as a relative equilibrium. Hence, I2 is interpreted as a steady state behavior found as a very parameter of the fatiguing trials. Moreover, the peak amplitude values of \( \text{SvO}_2 \) and rHb are interpreted as biological variability. This implies, that a homeostatic
adjustment of the oxygen saturation and blood filling during isometric actions would be possible despite a high intramuscular pressure, which was detected by other research groups and inferred as a cause of flow restriction.\(^{1,3,5}\)

**Measuring technique**

In contrast to other research groups we used the O2C device. The system unifies laser Doppler flowmetry and white light spectro photometry. Both techniques combined in the device meet the quality criteria. The advantage of the O2C device is the detection of rHb of only very thin venous vessels. Blood vessels with a diameter greater than 100 µm have no significant influence on the measurements. Because the high hemoglobin amount would absorb the light virtually completely\(^{20}\). In contrast, caused by the greater wavelength of the released light (700 to 900 nm), the NIRS method includes oxygen saturation of larger vessels (<1 mm diameter). The comparison with the inconclusive results from other studies has to be interpreted considering this fact.

**Results of oxygen saturation in comparison with other studies**

In the presented study we observed a phasic decrease during the MVIC tests particularly in type II, less than during the fatiguing trials at 60% MVIC. This could be explained by the shorter loading duration but is still speculative because of the low sample size. The MVIC tests lasted approximately 4 s compared to more than 40 s in the fatiguing trials. During such a short loading time a maximal deoxygenation might not be possible. The oxygen saturation in paravertebral muscles remained relatively stable after 30 s during an isometric trunk extension at 20% MVIC.

**Results of blood filling in comparison with other studies**

Regarding the hemoglobin concentration, most studies have described an immediate decrease and levelling off into a steady state behavior similar to our findings in type I\(^{10,11,21,24,25}\). Other research groups found an increase after an initial decrease\(^{11,17,18,25}\). In investigations with a shorter measuring time it is not clear whether the oxygen saturation would also have been adjusted into a steady state in their study. In colour fatiguing trials a long levelling off phase appeared if those trials only the first 30 s would be considered, the two different decay rates would be found, which were described by Felici and colleagues (2009). That is in line with findings of Jensen et al. (1999), in which the oxygen saturation in paravertebral muscles remain relatively stable after 30 s during an isometric trunk extension at 20% MVIC.

The two distinct types of regulation suggested in the present study do not seem to be person specific. Due to the small sample size we cannot give any statements about influencing factors such as gender although all female subjects were assigned to type I. The suggestion to categorize two behavioral patterns cannot exclude hybrid or transitional forms of regulation.

**Study limitations**

Like the NIRS technique, the O2C device is not able to exclude arterial blood completely.\(^{33}\) The tissue penetration of the white light of 12 mm does not represent the whole muscle. A statement about deeper regions, in
which the pressure on the microvessels might be higher,\textsuperscript{2,4} cannot be given. Caused by the pilot character of the study, some further limitations have to be considered in the interpretation of the results. It should be noticed, thatrometer was used for the exact determination of the elbow angle. Consequently, termination of loading was assessed by the examiner subjectively as soon as the elbow angle exceeded 90° for more than two seconds. Another is that the thickness of skin folds was not examined. Despite of this lack, the skin fold thickness on the anterior side of the upper arm is regularly low in normal weighted adults as participated here. Hence, low subcutaneous fat levels can be assumed. Moreover, in pretests different body types were compared. In a presence of an obvious thick subcutaneous fat layer, no change in the oxygenation during load occurred, i.e. the white light did not reach muscle tissue. Anyhow, results of the present study are worth to be replicated in both young and aged persons. The latter, may have peculiar circulatory impairments that will ask for special adaptations.

Conclusion
Based on the results of the current and previous studies, an adjustment of the oxygen saturation and relative hemoglobin amount into an isometric state during a fatiguing isometric action occurs at least in the superficial muscle layers. Maybe the blood flow in microvessels is not fully restricted due to the intramuscular pressure. The authors suggest to roughly categorize the behavior of muscle oxygenation and blood filling in two types. For a possible explanation, a triggered increase of the blood filling by threshold of the oxygenation level as a consequence of an intramuscular regulation is hypothesized. Further studies are necessary to understand the regulation mechanism.

List of acronyms
1\textsuperscript{st} Max. - first local maximum
1\textsuperscript{st} Min. - first local minimum
AU - arbitrary unit
BMI - body mass index
CV - coefficient of variation
I\textsubscript{1} - first interval
I\textsubscript{2} - second interval
MVIC - maximal voluntary isometric contraction
N\textsuperscript{1} D\textsuperscript{1}Ad\textsuperscript{1}e m\textsuperscript{1} - National Instruments D\textsuperscript{1}Ad\textsuperscript{1}e m\textsuperscript{1}
NIRS - nearinfrared spectroscopy technique
O\textsubscript{2}C - OxigenTo See LEA Medizintechnik GmbH
r\textsubscript{HB} - relative hemoglobin amount
RP - reversa point
S\textsubscript{v}O\textsubscript{2} - capillary-venous oxygen saturation of hemoglobin

Authors contributions
LS and FB have designed the study. SD has analyzed the data, has searched references and has written the manuscript. All authors were involved in the collection and interpretation of the data, revised the manuscript critically and done the final approval. They agree to be accountable for all aspects of the work.

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Conflict of Interest
The authors declare no potential conflict of interests.

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We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Muscle capillary behaviors during a fatiguing isometric action
Eur J Transl Myol 30 (1): xx1-xx9, 2020

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