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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 venous oxygen saturation of hemoglobin (SvO_2) decreased immediately after the start of loading and leveled off into a steady state. However, two different patterns (type I and type II) emerged. They differed in the extent of deoxygenation (–10.37 ±2.59 percent points (pp) vs. –33.86 ±17.35 pp, p = .008) and the behavior of the relative hemoglobin amount (rHb). Type I revealed a positive rank correlation of SvO_2 and rHb (ρ = 0.735, p <.001), whereas a negative rank correlation (ρ = –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

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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).[1-4] According to some authors, the resulted mechanical pressure might impede the blood flow within the muscle, which is essential for an adequate oxygen supply.[1-3,5] The blood flow could already be restricted at intensities of 15% MVIC.[2] 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.[6] 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 reflects the oxygen saturation of small veins (<1 mm diameter).[7] The principle and other limitations are described elsewhere.[7-9] 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.[10-17] This implies that the saturation stays nearly constant over time. On the other hand, it decreased continuously or in a phasic way with different decay rates until termination of the exercise.[17,19,20-25]

According to Sadamoto and colleagues (1983)[4] stopped outflow during isometric contractions due to a possible venous stasis should be considered, too. This could be verified by measuring the hemoglobin concentration as an indicator of the blood filling. In most cases, the total hemoglobin amount measured by the NIRS method decreased and behaved like the oxygen saturation.[10,11,21,24,25,27] However, there are also research groups which found an increase after an initial decrease,[17,20,28] or a direct increase after the start of load.[17,18,29] Inter-[19] and intra-individual differences were also found.[17]

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).[30] Thus, measurements mainly represent the capillary-venous oxygen saturation of hemoglobin (SvO_2) 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 foot or arterial occlusive diseases.[31,32]

This study questions how SvO_2 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 $\frac{kg}{m^2}$) 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.

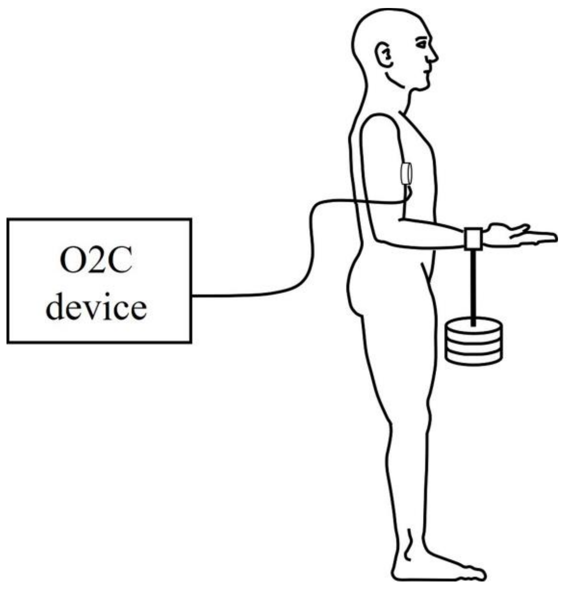

***Fig. 1** Measurement position and set up.*

Measuring technique

The non-invasive O2C device was utilized to record SvO_2 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 curve shape is more important than a quantitative comparison. The sampling rate was 40 Hz. Preliminary studies revealed that the device is valid, reliable,[31-33] and also applicable to muscle tissue at rest,[34] or during exercise.[35] 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 SvO_2 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.[36-38] During all measurements the room light was dimmed to minimize light effects on the probe. We monitored the parameters in the 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 double-sided adhesive film.

Setting and procedure

At the beginning the MVIC of each subject was determined. Six subjects pulled twice 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 four subjects should hold the highest possible weight for 1 s within maximal 5 steps. In both versions the arm position was identically to the subsequent fatiguing trial. For this, everyone had once 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, it provides 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 participant 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 (hereinafter 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.

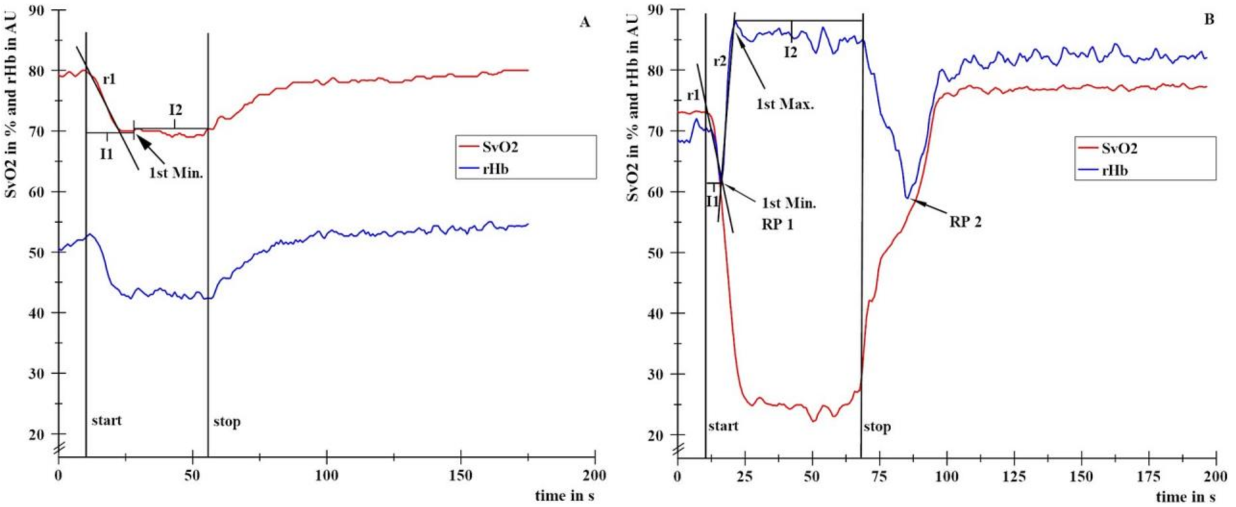

Fig 2. *Curve examples of the capillary-venous oxygen saturation of hemoglobin (SvO2) and the relative hemoglobin amount (rHb) in type I (**A**) and in type II (**B**) during a fatiguing trial of 60% of the MVIC. Start and stop of loading are indicated by vertical lines. The first (I1) and second (I2) interval, first local maximum (1st Max.) and minimum (1st Min.) or reversal points (RP1, RP2), respectively, and regression lines (r_1 and r_2) were set exemplarily in 2 curves. All curves are smoothed (moving average, maximal smoothing width: 50)*

Data processing and statistical analysis

The graphs of the raw data of the fatiguing trials and MVIC-tests were initially viewed in NI DIAdem™ 2012.

For further calculations all curves were smoothed (moving average, maximal smoothing width: 50). To describe and cluster the pattern of behavior of the parameters (SvO_2, rHb) the following variables were analyzed:

i. coefficient of variations (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 (I1) were set from start of loading to the following first local minimum (1st Min.), Figure 2 A, left panel. The boundaries of the second interval (I2) were set in two different versions depending on curve progression. If the 1st Min. is equivalent to a reversal point (RP), i.e. a direct continuous increase follows the first interval, the start of I2 was set at the first local maximum (1st Max.) after the continuous increase (Figure 2B, right panel). If a RP does not exist, the latter boundary of I1 (1st Min.) corresponds to the start of I2 (Figure 2A, left panel). In both versions, I2 ends at stop of loading.

To analyze i, arithmetic mean (M) and standard deviation (SD) of all data points within I2 were calculated. Subsequently, the CVs within one subject were computed ($\frac{SD}{M} \times 100$). Furthermore, peak-to-peak amplitudes of the parameters within I2 and their calculated means and SDs were extracted.

Variable ii was quantified by least square regression line within the I1 (r_1) and additionally, between I1 and I2 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 determined by calculating the differences from baseline values to the respective means of I2. They are presented in percent 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 (φ). 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 ± 68.57 N.

Based on curve shapes, the behavior of SvO_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 II. The BMI in type I was 21.89 ±2.54 $\frac{kg}{m^2}$ and 23.00 ±1.88 $\frac{kg}{m^2}$ in type II.

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

SvO_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 ±1.6 s. After the onset of recovery (after 49.72 ±12.35 s on average) both parameters approached to or increased above the baseline value, respectively.
The average CV of I2 within subjects was 1.19 ±0.75% in SvO_2 and 1.89 ±0.72% in rHb. The peak-to-peak amplitude of I2 within subjects amounted averagely 2.45 ±1.37 pp (min.–max.: 1.36–4.74) in SvO_2 and 3.39 ±1.14 AU (min.–max. 1.72–4.75) in rHb.
The slopes (ii) amounted averagely $r_1 = -1.69 \pm 0.92$ for SvO_2 and $r_1 = -1.83 \pm 0.47$ for rHb.
SvO_2 decreased by an average of –10.37 ±2.59 pp (≙ –13.38 ±2.75%) and rHb decreased averagely –11.17 ±6.3 AU (≙ –18.22 ±9.03%) below its baseline value.

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

SvO_2 and rHb showed a partial opposing behavior to each other, as illustrated in Figure 2B. SvO_2 decreased immediately with an average slope of $r_1 = -3.31 \pm 1.26$ and leveled off after averagely 15.55 ±3.23 s. At the onset of recovery (after 41.89 ±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 $r_1 = -2.65 \pm 0.88$. Directly after this turning point it increased with a slope of $r_2 = 4.81 \pm 1.55$ on average and leveled off at nearly the same time as SvO_2 did. During recovery rHb again decreased immediately to a second reversal point (RP 2) before it increased up to its baseline value or higher.
The average CV of I2 within one subject was 2.11 ±1.59% in SvO_2 and 1.41 ±0.75% in rHb. The peak-to-peak amplitude of I2 within subjects in SvO_2 and rHb amounted averagely 2.67 ±1.77 pp (min.–max.: 0.93–5.15) and 3.58 ±1.80 AU (min.–max.: 1.75–5.44), respectively.
SvO_2 decreased averagely –30.86 ± 17.35 pp (≙ –41.46 ± 22.4%). The rHb increased 9.03 ±10.48 AU (≙ 13.28 ± 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 sub-group of six subjects SvO_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 SvO_2 of both types 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 are a

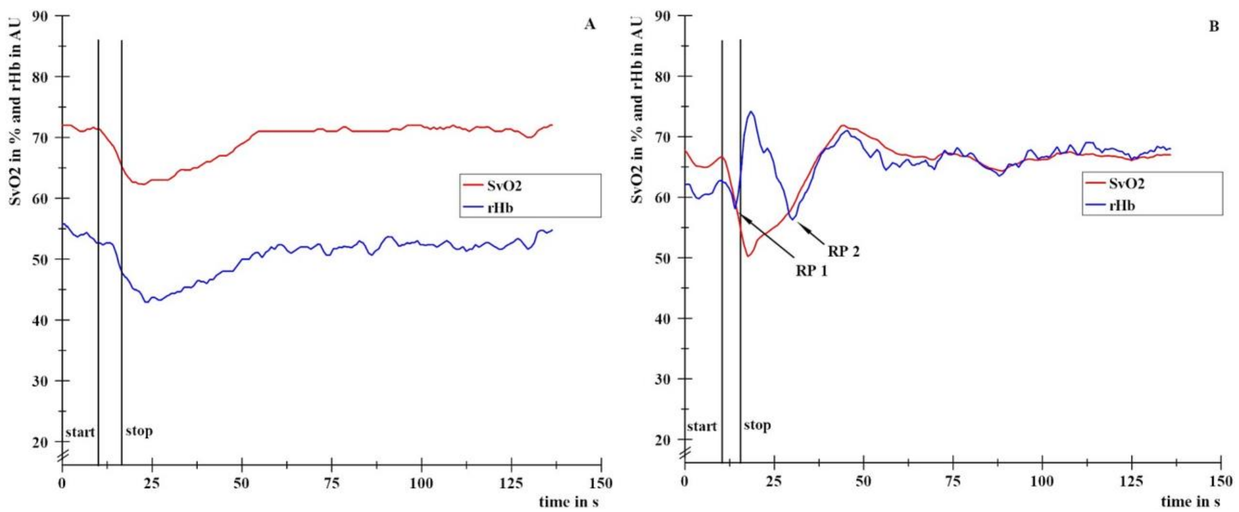

***Fig 3.** Curve examples of the capillary-venous oxygen saturation of hemoglobin (SvO_2) and the relative hemoglobin amount (rHb) in **type I (A)** and in **type II (B)** during a MVIC-test. Start and stop of loading are indicated by vertical lines. All curves are smoothed (moving average, maximal smoothing width: 50)*

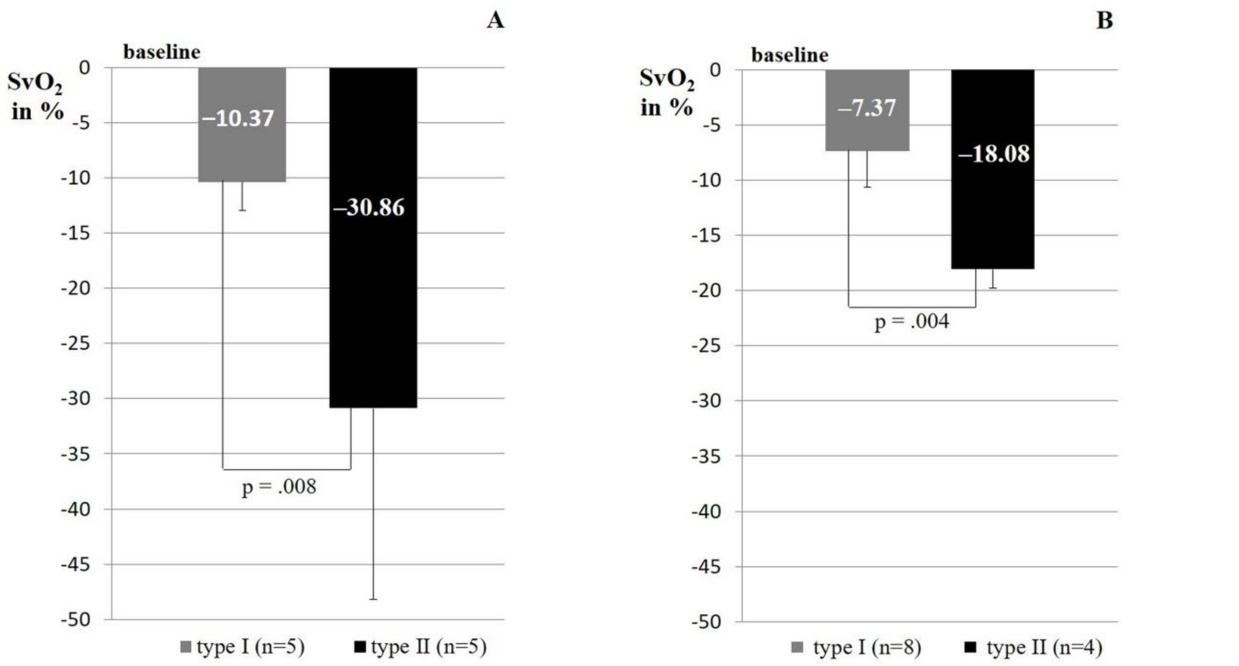

Fig. 4 *Means of the extent of deoxygenation during fatiguing trials at 60% of the maximal voluntary isometric contraction (MVIC) in type I (BCa 95%CI = –7.93 to –12.16) and type II (BCa 95%CI = –17.26 to –44.15) (**A**); and during MVIC-tests in type I (BCa 95%CI = –5.02 to –9.69) and type II (BCa 95%CI = –16.64 to –18.97) (**B**); Vertical lines express standard deviations.*

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 ($U = -2.61$, $p = .008$,

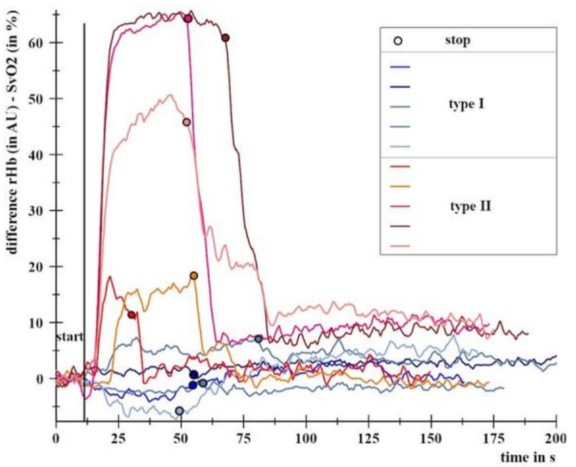

Fig 5. *Differences of the raw data between the relative hemoglobin amount (rHb) in AU and the capillary-venous oxygen saturation of hemoglobin (SvO_2) in % of the fatiguing trials (n = 10). All curves are smoothed (moving average, maximal smoothing width: 50)*

$\varphi = .37$) as well as in the MVIC-tests ($U = -2.72$, $p = 0.004$, $\varphi = .79$).

Concerning I2, type I had a positive rank correlation of SvO_2 and rHb from start until stop of loading with $\rho = 0.725$, $p < .001$ on average. In contrast, 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 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 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 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 in every parameter of the fatiguing trials. Moreover, the peak-to-peak amplitudes of values within I2 up to maximal 5.15 pp in SvO_2 and 5.44 AU in 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 spectrophotometry. Both techniques combined in the device meet the quality criteria.[31-33] The advantage of the O2C device is the detection of SvO_2 and rHb of only very thin venous vessels. Blood vessels with a diameter greater than 100 µm have no significant influence on the measurements. Because of the high hemoglobin amount, they would absorb the light virtually completely.[30] In contrast, caused by the greater wavelength of the released light (700 to 900 nm), the NIRS method includes the oxygen saturation of larger vessels (<1 mm diameter).[7] 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 SvO_2 decreased 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 SvO_2 of the MVIC-tests also showed no steady state behavior but a decrease to a reversal point and an immediate or a little delayed increase during recovery. This is comparable with findings of Maguire, Weaver and Damon (2007).[42] It might be necessary to sustain a load over a longer duration and consequently, submaximal intensities seem to be required in order to reach a steady state. Our results revealed durations between start of loading and leveling off into a steady state in both types of approximately 15 s. Studies which did not limit a loading duration and recorded muscle oxygenation until volitional fatigue are rare.[11,17,18,25] In investigations with a shorter measuring time, it is not clear whether the saturation would level off into a steady state or would decrease further on.

Fryer *et al.* (2014) observed a significantly higher extent of oxygen consumption measured by NIRS in elite climbing athletes compared to controls, intermediate and advanced groups.[25] In conclusion, the extent of deoxygenation might depend on the fitness level. However, in their study the oxygen saturation neither decreased to zero nor leveled off into a minimal physiological steady state until volitional fatigue occurred. Kell & Bhambhani (2008) also registered a continuous decrease of the muscle oxygenation albeit only after a slight increase.[18] The results of both studies mentioned above do not correspond to the findings of the presented study. We recorded an immediate decrease at the onset of loading and a leveling off into a steady state followed by an approach to or an increase above the baseline values when the loading was stopped. A steady state behavior was also found in studies with limited loading durations[10,12,16] and during fatiguing isometric muscle actions.[11,17] Irrespective of methodological differences it remains unclear, why a discrepancy relative to the two mentioned studies exists.

Felici *et al.* (2009) described a phasic decrease of the oxygen saturation of the biceps brachii muscle with two different decay rates until termination of the isometric action after 30 s of loading, regardless of the intensity (20–80% MVIC).[20] The initial phase had a fast and the second a slower decay rate, whereas the oxygen saturation in the slower phase proceeded very flat but still decreased.[20] This is comparable with the leveling off before adjusting a steady state as seen in the presented data. Because of the limited loading duration, it is unclear whether the oxygen saturation would also have been adjusted into a steady state in their study. In two of our fatiguing trials a long leveling off phase appeared. In 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).[20] 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.[4]

Results of blood filling in comparison with other studies

Regarding the hemoglobin concentration, most studies have described and / or graphed a decrease and leveling off into a steady state, similar to to our findings in type I.[10,11,21,24,25,27] Other research groups found an increase after an initial decrease,[20,28] or a direct increase at the start of loading.[17,29] This might fit to our findings in type II in the broadest sense. The study of Pereira and colleagues (2009) revealed the possibility of different behaviors in several subjects.[19] Furthermore, Akima and Ando (2017) found different behaviors in different muscles of the same individual.[17] 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 three female subjects were assigned to type II. 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.[7,9,43] 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,[2,44] 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, that no goniometer 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 one is that the thickness of skin folds were 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 a homeostatic steady 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 a 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

1st Max. - first local maximum
1st Min. - first local minimum
AU - arbitrary units
BMI - body mass index
CV - coefficient of variation
I1 - first interval
I2 - second interval
MVIC - maximal voluntary isometric contraction
NI DIAdem™ – National Instruments DIAdem™
NIRS - near infrared spectroscopy technique
O2C - Oxygen To See; LEA Medizintechnik GmbH
rHb - relative hemoglobin amount
RP - reversal point
SvO_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

Ethical Publication Statement

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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