

Effects of prolonged ischemia on human skeletal muscle microcirculation as assessed by near-infrared spectroscopy

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Abstract Near-infrared spectroscopy (NIRS) has been used to detect in vivo microvascular alterations by means of a vascular occlusion test. We sought to analyse by NIRS the microcirculatory profile of patients undergoing prolonged tourniquet-induced bloodless condition for extremity surgery, and compare the results with time of ischemia and comorbidities. We conducted a prospective observational study on 42 patients undergoing upper limb surgery. Regional anaesthesia was achieved and ischemia was induced by a tourniquet cuffed at 250 mmHg. The probe of a NIRS monitor (InSpectra 325, Hutchison, USA) was placed on the brachial muscle, and muscle oxygen saturation (StO₂) was recorded continuously before anaesthesia, during and after surgery. The following variables were recorded: baseline StO₂, StO₂ desaturation slope during occlusion (dSlope, units/s), resaturation rate following ischemia (RR, units/s), hyperaemic peak (peak, units), and duration of the hyperaemic period following ischemia (hyperaemic time, s). Values of dSlope were similar among all patients. RR and hyperaemic time were significantly correlated with the duration of ischemia, but not with comorbidities [$p = 0.007$ CI (−35.64 to −13.1), and $p < 0.001$ CI (0.049–0.159), respectively]. Grouping patients by duration of ischemia (30, 60, or 90 min), we found a significant decrease in RR after 60 and 90 min

($p < 0.001$ and $p = 0.03$, respectively). Hyperaemic peak was lower in the 90 min group (83.9 ± 6.8 vs. 91.2 ± 5.7 %, $p = 0.02$) whereas the hyperaemic time was significantly increased (595 ± 136 vs. 429 ± 107 min, $p < 0.001$). Alterations of skeletal muscle microcirculation were correlated with the duration of ischemia, but not with comorbidities. We observed an initial impairment of the microcirculatory recovery at 90 min of ischemia.

Keywords Microcirculation · Spectroscopy · Near-infrared · Muscle · Skeletal · Ischemia

1 Introduction

Alterations of skeletal muscle tissue and its microvascular ultrastructure after ischemia have been investigated in animal models in vivo by intravital microscopy and in vitro through biological, histological, and anatomical analyses [1]. Although these experimental studies have provided information on the no-flow phenomenon in ischemia and reperfusion injury [2], few studies have focused on microvascular alterations in humans after prolonged ischemia [3, 4].

Near-infrared spectroscopy (NIRS) is widely recognized as a non-invasive method to monitor oxygen saturation of tissue (StO₂) [5]. In recent years, StO₂-derived variables obtained by means of an arterial vascular occlusion test (VOT) have been used to evaluate the microcirculatory profile of patients with various clinical conditions [6–9]. To study the effects of prolonged ischemia on skeletal muscle microcirculation in humans using noninvasive methods, we assessed microvascular reperfusion with NIRS in orthopedic hand surgery procedures that required tourniquet-induced bloodless conditions. We hypothesized that

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microcirculatory reperfusion after prolonged ischemia is affected by the duration of ischemia and/or the presence of cardiovascular comorbidities.

2 Methods

2.1 Study population

This prospective observational study was performed in the Department of Orthopaedics and Traumatology at Careggi University Hospital, Florence (Italy). The local Ethical Committee approved the study (protocol no. 104/13), and informed consent was obtained from all participants. According to study protocol all patients scheduled for hand surgery that required tourniquet for prolonged bloodless surgical condition were enrolled between August and September 2013 (4 months of observation time). Patients who were less than 18 years old or had undergone general anesthesia for the procedure were excluded from participation.

2.2 Near-infrared spectroscopy

NIRS technology is described in detail elsewhere [10, 11]. Briefly, the near-infrared light emitted from the probe of a spectrometer is partially absorbed by chromophores (mainly hemoglobin [10]) and reflected from the tissue back to the probe. The collected absorbance values provide a measure of oxyhemoglobin (HbO₂) and deoxyhemoglobin (HHb) contained in small vessels (diameter less than 1 mm according to Beer–Lambert's law) of epidermis, subcutaneous adipose tissue, and skeletal muscle [12]. Although the relative contribution of arterial, capillary, and venous blood cannot be determined using this method, the capillary and venous segments are estimated to represent 90 % of the microvascular signal [13]. Both HbO₂ and HHb were used to calculate tissue oxygen saturation:

$$\text{StO}_2 = [\text{HbO}_2]/([\text{HHb}] + [\text{HbO}_2]) \%$$

In recent years, a brief (3–5 min) arterial vascular occlusion test (VOT) during StO₂ monitoring has been proposed in order to assess local metabolic demand and microvascular reactivity of the skeletal muscular tissue [6, 14]. In particular, the descending slope of StO₂ reflects oxygen diffusion from microvessels to cells during stagnant ischemia (i.e. when metabolic demand and oxygen consumption are equivalent) [15]. Ascending slope of StO₂ represents the reperfusion phase after an ischemic challenge and depends on the ratio between oxygen delivery and oxygen consumption (DO₂/VO₂), microvascular recruitment, and compliance. The ascending slope is an indicator of microvascular reactivity, along with the

variables obtained from the hyperemic phase (StO₂ peak after ischemia and the duration of the hyperemic period) [5, 14, 16].

In the present study, the distance between illumination and detection fibers of the used probes was 25 mm, which yielded a signal acquisition depth of up to 23 mm [17].

2.3 Clinical management

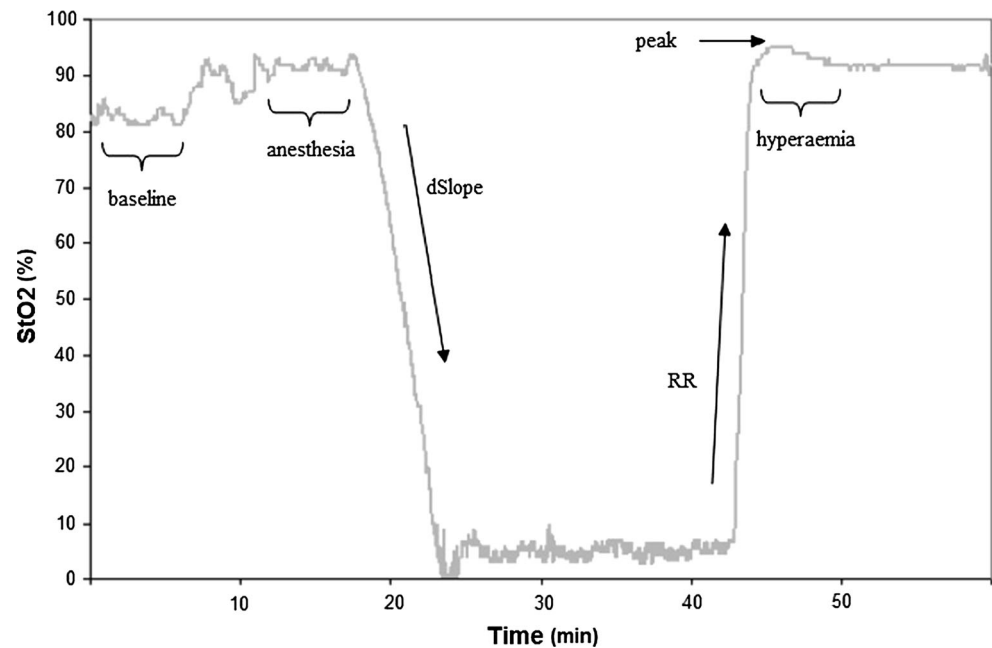
All subjects underwent surgery in the same operating room with temperature set at 21 °C. Regional anesthesia was achieved 30 min before ischemia with a complete echo-guided axillary brachial plexus block, infiltrating 150 mg of 0.5 % levobupivacaine as standard care. Ischemia was induced by a pneumatic tourniquet (Zimmer ATS 2000, Zimmer Inc., Warsaw, IN, USA) cuffed at 250 mmHg within 3 s and released within 1 s at the end of surgery. During surgery, patients received minimal sedation with an intravenous bolus of 1 mg midazolam every 30 min and were breathing spontaneously in ambient air. Continuous ECG, non invasive blood pressure (NIBP), heart rate (HR) and peripheral oxygen saturation (SpO₂) were routinely monitored.

2.4 Data collection

Demographics, pre-existing chronic diseases, and intraoperative parameters were recorded. The probe of a NIRS monitor (InSpectra 325, Hutchinson Technology Inc., Hutchinson, MN, USA) was placed preoperatively at the brachioradialis muscle of the arm to be operated on, and StO₂ monitoring started. StO₂ was recorded continuously before anesthesia and during and after surgery, and the following were analyzed with a dedicated software (InSpectra Analysis Software V.4.03): before anesthesia (baseline, %units), after anesthesia (anesthesia, %units) the difference between before and after (deltaStO₂ %units), the desaturation slope during occlusion (dSlope, units/s), the resaturation rate of the reperfusion phase after the ischemic period (RR, units/s), the hyperemic peak (peak, %units), and duration of the hyperemic period following ischemia (hyperemic time, s) (Fig. 1). Slope values were calculated within the range of values from 30 to 100 % of StO₂.

2.5 Statistical analysis

Statistical analyses were performed using the SPSS 18.0 for Windows XP software package (SPSS Inc. 2004, Chicago, USA) and GraphPad (Vers. Prism 4.0; San Diego, California). To assess normal distribution of data, a Kolmogorov–Smirnov test was performed. Discrete variables were expressed as counts (percentage) and continuous variables as mean ± SD or median with interquartile

Fig. 1 StO₂-derived variables

ranges where applicable. To verify the association between outcomes (dSlope, RR, peak, hyperemic time) and covariates (duration of ischemia, age, sex, BMI, presence of diabetes, hypertension, cardiopathy, COPD, vasculopathy, autoimmune disease, neoplastic disease, tabagism, and intraoperative physiological parameters), a multivariate linear regression with stepwise variables selection was used, and 95 % confidence intervals (CI) were computed (Table 1). To assess variance, a Bartlett test was used to perform one-way ANOVA. Groups were compared using a Tukey's test for multiple comparisons or Fisher's exact test as appropriate. Significance was set at $p < 0.05$.

3 Results

Of the 57 patients admitted for hand surgery over the study period, 41 met the inclusion criteria ($n = 10$ underwent general anesthesia, $n = 6$ less than 18 years old) and 39 underwent final analysis ($n = 2$ missing data due to unreliable signal acquisition). Patient characteristics and intraoperative physiological parameters are shown in Table 2, and NIRS-derived variables are shown in Table 3.

Baseline StO₂ was (mean $\pm$ SD) 70.1 ± 15.3 % and increased significantly to 82 ± 12.6 % after locoregional anesthesia ($p < 0.001$). DeltaStO₂ was significantly associated with the presence of diabetes and neoplastic disease [$p = 0.024$ CI (0.003–0.028) and $p = 0.004$ CI (0.001–0.033), respectively] due to the lower baseline StO₂ in these groups of patients (diabetes, 46.8 ± 15 vs. 73.9 ± 12.2 % for patients

with no comorbidities, both $p < 0.001$). The dSlope was not correlated with demographics nor comorbidities (Table 1). The RR and recovery time were significantly associated with the duration of ischemia [$p = 0.007$ CI (–35.64 to –13.1), and $p < 0.001$ CI (0.049–0.159), respectively], but not with other covariates (Fig. 2; Table 1). These relationships were linear and no inflection point to indicate a critical threshold was found. Grouping patients by duration of ischemia (30, 60, or 90 min corresponding to A, B, and C groups, respectively), we found a significant decrease in RR (A vs. C, $p < 0.001$ and B vs. C, $p = 0.03$) and increase in recovery time (A vs. C, $p < 0.001$ and B vs. C, $p = 0.025$) after 90 min of ischemia (Table 3; Fig. 3). The hyperemic peak was also lower in the C group than in the B group ($p = 0.02$) (Table 3; Fig. 3). After the hyperemic period, StO₂ returned to pre-ischemic values in all patients.

4 Discussion

Current knowledge of the alterations of microvascular structure and function after prolonged ischemia is mainly based on animal models [18]. To our knowledge, this was the first study to investigate in vivo the effects of prolonged ischemia and reperfusion on human skeletal muscle microcirculation, as assessed by NIRS. We utilized the hand surgery operative setting, which required prolonged bloodless conditions, to conduct this study. Locoregional anesthesia and minimal sedation guaranteed patient comfort during data collection.

Table 1 Multivariate analysis to identify correlations between outcomes and covariates

	Outcomes									
	delta StO ₂ (%units)		dSlope (units/min)		RR (units/s)		Peak (%units)		Recovery time (s)	
	95 % CI	p value	95 % CI	p value	95 % CI	p value	95 % CI	p value	95 % CI	p value
<i>Covariates</i>										
Duration of ischemia (min)	–	–	–	–	–35.640	–13.100	–2.287	0.428	0.049	0.159
Age (years)	0.402	2.017	0.101	0.131	0.752	18.290	–1.772	0.001	–0.030	0.057
Sex	–0.035	0.011	0.371	0.337	–0.283	0.200	–0.007	0.040	–0.001	0.001
BMI (kg/m ²)	–0.016	0.320	0.074	0.298	–1.940	1.680	–0.244	0.117	–0.003	0.016
Diabetes	0.003	0.028	0.024	0.362	–0.083	0.194	–0.025	0.002	0.000	0.001
Hypertension	–0.007	0.051	0.181	0.381	–0.110	0.376	–0.054	0.009	–0.001	0.002
Cardiopathy	–0.007	0.020	0.307	0.646	–0.159	0.120	–0.006	0.021	–0.001	0.000
COPD	–0.007	0.020	0.351	0.520	–0.099	0.179	–0.010	0.018	–0.001	0.000
Vasculopathy	–0.004	0.012	0.282	0.777	–0.077	0.089	–0.006	0.011	–0.001	0.000
Autoimmune disease	–0.010	0.020	0.501	0.164	–0.194	0.123	–0.017	0.015	–0.001	0.001
Neoplastic disease	0.001	0.033	0.004	0.311	–0.128	0.221	–0.022	0.013	–0.001	0.000
Tabagism	–0.024	0.021	0.694	0.576	–0.313	0.142	–0.028	0.018	–0.001	0.001
SpO ₂ (%)	–0.020	0.135	0.14	0.676	–1.377	0.221	–0.087	0.077	–0.003	0.005
BPsys (mmHg)	–0.276	1.631	0.158	0.464	–1.057	18.350	–1.746	0.218	–0.032	0.062
BPdia (mmHg)	–0.429	0.645	0.686	0.363	–1.890	8.978	–0.545	0.570	–0.045	0.005

Bold values indicate $p < 0.05$

BMI, body mass index; COPD, chronic obstructive pulmonary disease; Bsys, systolic arterial pressure; Bdia, diastolic arterial pressure; dSlope, descending slope; RR, recovery rate

Table 2 Patient characteristics and intraoperative physiological parameters

	Overall	A (n = 18)	B (n = 12)	C (n = 9)	p value
Duration of ischemia (minutes, <i>mean ± SD</i>)	52.2 ± 26.2	29.6 ± 8.4* [†]	58 ± 6.7* [‡]	89.6 ± 16.4 ^{†‡}	<0.001
Age (years, <i>mean ± SD</i>)	51.7 ± 17.4	52.4 ± 20	51.7 ± 11	50.5 ± 20	0.968
Male [n(%)]	29 (69)	13 (72)	8 (66)	6 (66)	0.932
BMI (kg/m ² , <i>mean ± SD</i>)	25.4 ± 3.5	25.4 ± 3	25.5 ± 5	25.5 ± 3	0.984
Comorbidities [n(%)]					
Diabetes	4 (9.5)	3 (16.6)	0 (0)	1 (11.1)	0.336
Hypertension	13 (31.0)	6 (33.3)	3 (25)	2 (22.2)	0.676
Cardiopathy	3 (7.1)	1 (5.5)	1 (8.3)	1 (11.1)	0.853
COPD	3 (7.1)	1 (5.5)	1 (8.3)	1 (11.1)	0.853
Vasculopathy	1 (2.4)	0 (0)	1 (8.3)	0 (0)	0.302
Autoimmune disease	5 (11.9)	1 (5.5)	2 (16.6)	2 (22.2)	0.391
Cancer	6 (14.3)	5 (27.7)	1 (8.3)	0 (0)	0.121
Tabagism	12 (28.6)	6 (33.3)	3 (25)	3 (33.3)	0.873
Intraoperative variables (<i>mean ± SD</i>)					
Bsys (mmHg)	140.6 ± 19	140.8 ± 16	138.7 ± 19	133.9 ± 20	0.653
Bdia (mmHg)	74.1 ± 10.7	75.5 ± 11	70.6 ± 8.8	70.5 ± 8	0.3
SpO ₂ (%)	96.9 ± 1.7	96.3 ± 1.2	97.4 ± 2	97.5 ± 1.8	0.12

BMI, body mass index; COPD, chronic obstructive pulmonary disease; Bsys, systolic arterial pressure; Bdia, diastolic arterial pressure

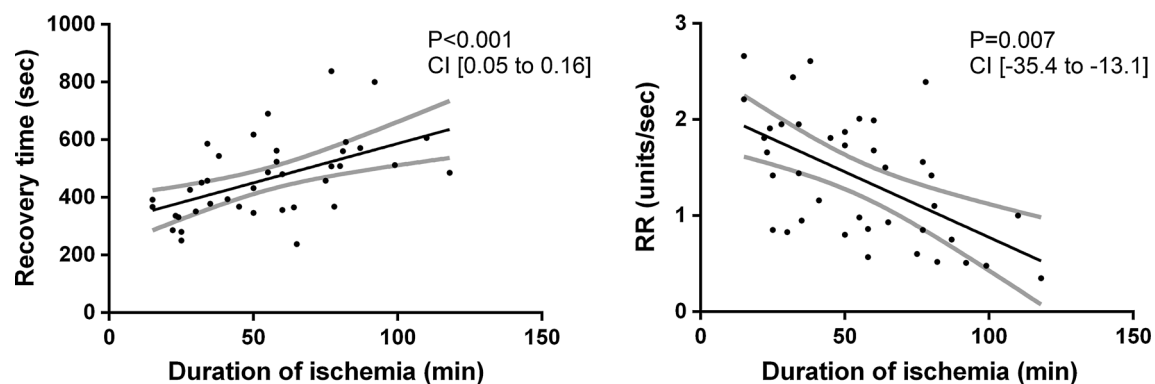
* significant versus C, [†] significant versus B, [‡] significant versus A

Table 3 StO₂ derived variables

StO ₂ variables	Overall (n = 39)	A (n = 18)	B (n = 12)	C (n = 9)
Baseline (%units)	70.1 ± 15.3	71.6 ± 13.5	69.1 ± 19	68 ± 15
Anesthesia (%units)	82 ± 12.6	83.5 ± 10.6	81.2 ± 15.5	81.2 ± 13.3
deltaStO ₂ (%units)	12 ± 6.8	11.8 ± 7.9	12 ± 6.8	13.2 ± 4.8
dSlope (units/min)	−13 ± 4.4	−13 ± 4.6	−12.5 ± 3.3	−13.9 ± 4.2
RR (units/s)	1 ± 0.6	1.7 ± 0.6	1.4 ± 0.5	0.7 ± 0.3* [†]
Peak (%units)	89 ± 6.3	89 ± 5.5	91.2 ± 5.7	83.9 ± 6.8 [†]
Recovery time (s)	441 ± 109	387 ± 88	459 ± 127	595 ± 136* [†]

dSlope, desaturation slope; RR, resaturation rate; Peak, hyperemic peak

* significant versus A, [†] significant versus B

**Fig. 2** Correlations between duration of ischemia and recovery time (*left*) and resaturation rate (*right*)

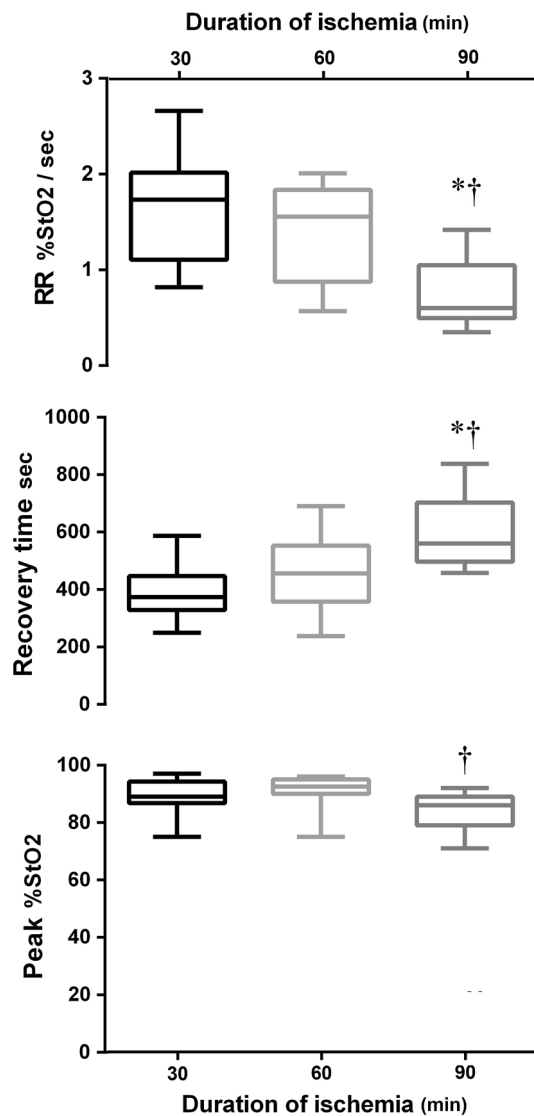


Fig. 3 Resaturation rate, recovery time, and hyperemic peak of subject grouped by duration of ischemia

The perioperative monitoring of StO_2 provided a variety of dynamic variables that described the microvascular profile during and after prolonged ischemia. We observed a significant increase in StO_2 after the onset of locoregional anesthesia. This was expected because brachial plexus block causes functional sympathectomy-like effects that lead to a complete vasodilatation and a subsequent increase in blood flow, microvascular arteriovenous shunting, and ultimately an increase in oxygen saturation [19, 20].

This effect was more pronounced in subjects with diabetes mellitus or a history of neoplastic disease treated with chemotherapy. Microangiopathy has been observed with both of these conditions [21–24], and the pronounced increase in StO_2 after functional sympathectomy should be further investigated.

The dSlope was similar among all patients, which agrees with previous studies that show dSlope is similar between healthy volunteers and hospitalized control groups (with the exception of critically ill patients with sepsis) [6, 7]. The dSlope represents the rate of oxygen diffusion from the microvessels to the cells during stagnant ischemia and depends on endothelial integrity and tissue metabolic demand [25]. Assuming normal capillary oxygen diffusion and a similar metabolic activity in our cohort of patients, these results were not surprising.

Alterations in microvascular reperfusion parameters after prolonged tourniquet-induced ischemia were significantly associated with the duration of ischemia and not with others covariates such as demographics, intraoperative physiological parameters, or pre-existing comorbidities. In particular, the RR decreased proportionally with increased duration of ischemia. RR is an indicator of microvascular reserve [26] and should not be confused with the concept of perfusion. Specifically, the StO_2 upslope represents the removal of HHb from the ischemic skeletal muscle in conjunction with the influx of HbO_2 and is not a measure of blood flow *per se*. Changes in StO_2 may be directly proportional to a change in flow and inversely related to changes in metabolism [27]. However, assuming a constant basal metabolism in locally anesthetized subjects, we hypothesize that the decrease in RR was primarily related to a progressive reduction in microvascular blood flow.

The hyperemic peak was also lower in patients with longer duration of ischemia (Fig. 3). The hyperemic peak reflects the microvascular recruitment during reactive hyperemia caused by nitric oxide and prostacyclin and should be higher with longer duration of ischemia. However maximal vasodilatory response is achieved within minutes of stagnant ischemia [28]. Moreover, because the microvascular tree is maximally dilated and accessible for perfusion after ischemia, the lower hyperemic overshoot in patients with longer duration of ischemia was likely due to reduced microvascular conductance, as evidenced by the reduction in RR.

Finally, the recovery time, which represents the ability of microvessels to recover from an ischemic challenge, was significantly longer in patients with a longer duration of ischemia. Recovery time is considered an indicator of microvascular compliance [29] and is affected by local blood flow, metabolic imbalance, microvascular ultra-structure, and oxygen consumption. The increased ischemia recovery time may be due to reduced capillary blood flow and substantial damage of capillary fine structure.

Although we did not find any critical threshold for ischemia duration, we observed significant changes in reperfusion parameters in patients who underwent surgery for approximately 90 min. As observed in several animal studies, the “no-reflow phenomenon”, defined as post-

ischemic microvascular failure due to microvessel occlusion, is time dependent and the primary reason for necrosis of skeletal muscle tissue after prolonged ischemia [30]. Several studies show that 3–4 h of ischemia results in irreversible microvascular reperfusion injury due to impaired integrity of capillary endothelial cells, as evidenced by vacuolization and swelling accompanied by microthrombosis and hemolysis of intraluminal erythrocytes [31, 32]. In particular, prolonged ischemia impairs the integrity of the capillary endothelium, which increases microvascular permeability and results in intravascular hemoconcentration and reduced capillary conductance due to interstitial edema [33, 34]. Moreover, endothelial cell swelling [35, 36], expression of adhesion molecules leading to leukocyte capillary plugging [37, 38], and local activation of coagulation with microthrombosis all contribute to the aggravation of ischemic injury either by the protraction of ischemia (no-reflow) [1] or by the action of cytokines, oxygen metabolites, and complement cascade factors (reflow-paradox) [39]. All of these alterations have been observed in animal models in which duration of ischemia was the main determinant of such histological damage [40–42]. Furthermore, a human study by Gidlöf et al. [3] that conducted histological analyses of skeletal muscle biopsies during and after prolonged ischemia (median 110 min) showed a progressive impairment of capillary endothelial cells in patients undergoing knee surgery.

Use of a tourniquet is common in orthopedic surgery because it controls intraoperative blood loss and improves visualization of the surgical field. The safety of this technique is still controversial, as it has been associated with numerous postoperative complications [43]. However, an arbitrary time limit of 2 h, derived from studies on skeletal muscle metabolism and ultrastructure after prolonged ischemia, is commonly accepted in orthopedic theatres [44, 45] and is considered to be safe in the absence of clinical postoperative complications [46].

Our findings showed initial alterations in microvascular function occurred slightly earlier, which may be explained by the fact that in vivo analysis of the microvascular profile, as assessed by NIRS, shows physiological alterations in microvascular function that precede the permanent modifications of capillaries observed in the histological analyses. Indeed, StO₂ returned to pre-ischemic values in all patients, indicating that a functional recovery of microvessels was attained even after 90 min of stagnant ischemia.

Some limitations must be taken into account. The variability of vasomotor tone and local blood volume in the peripheral circulation are to some extent unpredictable, and their effect on StO₂-derived parameters are not fully understood. In particular, fluctuations in peripheral vasomotor tone could affect the NIRS measurements [47].

Although the skin temperature was not directly measured to estimate variations in peripheral blood flow, we specifically maintained a constant room temperature of 21 °C. In addition, we used a 25 mm probe that yielded a signal acquisition depth of up to 23 mm and, therefore, could overcome the effect of superficial layers vasomotion. Another limitation of the method is the low sensibility of NIRS for low StO₂ values [11]. After a protracted time of tourniquet-induced ischemia, we observed an unexpected drop of StO₂ levels below 20 %. This instance could theoretically affect the values of both descending and ascending StO₂ slopes. However, this finding did not affect our observations as slopes were calculated for a range of StO₂ values between 100 and 30 %.

In conclusion, alterations of human skeletal muscle microcirculation after prolonged ischemia were associated with duration of ischemia but not with demographics, intraoperative physiological parameters, or pre-existing comorbidities. These alterations were more pronounced in patients undergoing prolonged (90 min) stagnant ischemia.

Compliance with ethical standards

Conflict of interest The authors report no conflict of interest concerning the elaboration of this work.

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