

Increased tissue oxygenation explains the attenuation of hyperemia upon repetitive pneumatic compression of the lower leg

*Original*

Increased tissue oxygenation explains the attenuation of hyperemia upon repetitive pneumatic compression of the lower leg / Messere, Alessandro; Ceravolo, Gianluca; Franco, Walter; Maffiodo, Daniela; Ferraresi, Carlo; Roatta, Silvestro. - In: JOURNAL OF APPLIED PHYSIOLOGY. - ISSN 8750-7587. - 123:6(2017), pp. 1451-1460. [10.1152/jappphysiol.00511.2017]

*Availability:*

This version is available at: 11583/2696430 since: 2018-01-09T10:41:51Z

*Publisher:*

American Physiological Society

*Published*

DOI:10.1152/jappphysiol.00511.2017

*Terms of use:*

This article is made available under terms and conditions as specified in the corresponding bibliographic description in the repository

*Publisher copyright*

(Article begins on next page)

## RESEARCH ARTICLE

# Increased tissue oxygenation explains the attenuation of hyperemia upon repetitive pneumatic compression of the lower leg

Alessandro Messere,<sup>1</sup> Gianluca Ceravolo,<sup>2</sup> Walter Franco,<sup>2</sup> Daniela Maffiodo,<sup>2</sup> Carlo Ferraresi,<sup>2</sup> and  Silvestro Roatta<sup>1</sup>

<sup>1</sup>Department of Neuroscience, University of Torino, Turin, Italy; and <sup>2</sup>Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Turin, Italy

Submitted 31 May 2017; accepted in final form 15 August 2017

**Messere A, Ceravolo G, Franco W, Maffiodo D, Ferraresi C, Roatta S.** Increased tissue oxygenation explains the attenuation of hyperemia upon repetitive pneumatic compression of the lower leg. *J Appl Physiol* 123: 1451–1460, 2017. First published August 17, 2017; doi:10.1152/jappphysiol.00511.2017.—The rapid hyperemia evoked by muscle compression is short lived and was recently shown to undergo a rapid decrease even in spite of continuing mechanical stimulation. The present study aims at investigating the mechanisms underlying this attenuation, which include local metabolic mechanisms, desensitization of mechanosensitive pathways, and reduced efficacy of the muscle pump. In 10 healthy subjects, short sequences of mechanical compressions ( $n = 3–6$ ; 150 mmHg) of the lower leg were delivered at different interstimulus intervals (ranging from 20 to 160 s) through a customized pneumatic device. Hemodynamic monitoring included near-infrared spectroscopy, detecting tissue oxygenation and blood volume in calf muscles, and simultaneous echo-Doppler measurement of arterial (superficial femoral artery) and venous (femoral vein) blood flow. The results indicate that 1) a long-lasting ( $>100$  s) increase in local tissue oxygenation follows compression-induced hyperemia, 2) compression-induced hyperemia exhibits different patterns of attenuation depending on the interstimulus interval, 3) the amplitude of the hyperemia is not correlated with the amount of blood volume displaced by the compression, and 4) the extent of attenuation negatively correlates with tissue oxygenation ( $r = -0.78$ ,  $P < 0.05$ ). Increased tissue oxygenation appears to be the key factor for the attenuation of hyperemia upon repetitive compressive stimulation. Tissue oxygenation monitoring is suggested as a useful integration in medical treatments aimed at improving local circulation by repetitive tissue compression.

**NEW & NOTEWORTHY** This study shows that 1) the hyperemia induced by muscle compression produces a long-lasting increase in tissue oxygenation, 2) the hyperemia produced by subsequent muscle compressions exhibits different patterns of attenuation at different interstimulus intervals, and 3) the extent of attenuation of the compression-induced hyperemia is proportional to the level of oxygenation achieved in the tissue. The results support the concept that tissue oxygenation is a key variable in blood flow regulation.

muscle blood flow; hyperemia; muscle compression; tissue oxygenation

SINCE THE SEMINAL WORK of Mohrman and Sparks (39) several studies have demonstrated that a rapid and transient hyperemic response can be elicited by a short-lasting muscle compression

(10, 30, 38, 56–60). Although the underlying mechanisms have not been fully identified, this phenomenon has been well documented in different experimental models, such as the isolated muscle (39), awake and anesthetized animals (57, 58, 60), and humans (10, 30, 38, 56). In addition, a rapid dilatory response to compressive stimuli has also been observed in isolated feed arteries (7). More controversial is the hemodynamic response to repeated compressive stimuli. Kirby et al. (30) observed that the response to five consecutive compressions was nonsignificantly attenuated with respect to the response to a single compression. Conversely, Clifford et al. (7), using the same pattern of five consecutive compressive stimuli on an isolated muscle feed artery, observed a significant increase of the dilatory response compared with the single compression.

In a recent work, Turturici and Roatta investigated the blood flow response to a longer-lasting sequence of mechanical stimulations (20 compressions, 1 s on/1 s off) reporting that the initial hyperemic response progressively fades away in spite of continuing stimulation and hypothesized that the mechanosensitive mechanism underlying the response could undergo some kind of transient inactivation (60). In fact, the attenuation of the compression-induced hyperemia was observed to increase at increasing stimulation frequencies (60). A similar behavior was recently observed also in humans (38).

Surprisingly, this phenomenon has been poorly described in the several investigations concerning the hyperemic effect of intermittent pneumatic compressions (IPC; 14, 15, 32–34) and in experimental studies investigating the mechanisms underlying compression and contraction-induced hyperemia (9, 24, 31, 40, 44), with the exception of a short report by Tschakovsky et al. (56). In this pioneering investigation the authors observed that repetitive compression of the forearm below heart level exhibited a transient hyperemia settling to a lower level after 10–20 s from the beginning of the treatment (56). More recently, Sheldon et al. (47) also reported attenuation of the hyperemia during IPC treatment, although on a larger time-scale (45 vs. 5 min from the beginning), and observed that the effect was dependent on the frequency of stimulation.

The issue is relevant because improving limb perfusion is a major aim in the treatment of disorders such as peripheral arterial disease and is pursued in sport medicine for accelerated recovery from fatigue (1, 35). Understanding of the underlying mechanisms is essential for implementing optimal treatments (46).

Address for reprint requests and other correspondence: S. Roatta, Dip. di Neuroscienze, Università degli Studi di Torino, Corso Raffaello 30, 10125 Torino, Italy (e-mail: silvestro.roatta@unito.it).

Potential mechanisms underlying attenuation of the hyperemia during repetitive mechanical stimulation include 1) inactivation of the mechanosensitive vasodilatory pathways (60), 2) diminished efficacy of the muscle pump (56), and 3) local regulatory mechanisms that may be activated in response to hyperperfusion (30, 56). Unfortunately, none of these possibilities is supported by solid experimental evidence. In particular, 1) mechanosensitive channels exhibiting inactivation properties have been identified (17, 26), but their actual involvement in the rapid compression-induced dilatation was not ascertained; 2) at high stimulation frequencies, incomplete vascular refilling may reduce the contribution of the pump, but a role for the muscle pump was excluded in a previous animal study (60); and 3) local vasoconstrictory mechanisms are known to act in response to hyperperfusion, but little is known about the actual regulatory variable ( $O_2$ ,  $CO_2$ , pH, etc.) and about the strength and timing of this vascular reaction (6, 45). However, in a recent reformulation of the metabolic control of blood flow, a primary role for tissue  $PO_2$  has been postulated (23). According to their model, an excessive rise in  $O_2$  concentration within the tissue would trigger a vasoconstrictory response, mediated by the inhibition of a tonically released vasodilator (23). Along this line, a rise in tissue  $O_2$  occurring during compression-induced hyperemia could then trigger a constrictor response and limit further hyperemic events in response to subsequent mechanical stimuli.

On this basis, the present study aimed to test the following hypotheses: 1) compression-induced hyperemia elicits a rise in tissue oxygenation; 2) the attenuation of the hyperemic response to subsequent compressive stimuli is related to the extent of hyperoxygenation achieved in the tissue; and 3) other mechanisms, namely, the intrinsic inactivation of mechanosensitive pathways and the muscle pump, would have a minor role in the attenuation of the hyperemic response upon repetitive compressive stimulation.

To assess changes in tissue oxygenation, near-infrared spectroscopy (NIRS) was adopted. By locating the NIRS probe under the compressive cuff, continuous monitoring of local oxygenation and blood volume changes from the relevant muscles was achieved. Moreover, in addition to arterial inflow, venous outflow was also monitored as its response to the compression is an indicator of the extent of filling of the venous compartments and thus of the efficacy of the muscle pump exerted by compressive stimuli.

## MATERIALS AND METHODS

**Ethical approval.** Ten healthy subjects (8 men and 2 women; age,  $27.1 \pm 3.0$  yr; weight,  $67.9 \pm 11.7$  kg; height,  $176.7 \pm 9.7$  cm, means  $\pm$  SD) were recruited for the present study. All subjects were normotensive and nonobese. The study conformed to the standards set by the Declaration of Helsinki and was approved by the Local Ethical Committee (protocol no. 60195), and all subjects gave their written informed consent after they were instructed about purpose and procedures of the experiment.

**Mechanical leg compressions.** A previously tested prototype of the IPC device was employed in the present study to deliver controlled and repeatable compressions to the leg of the subject (19, 20). Briefly, the device exerts a compressive action by inflating five different bladders wrapped around the foot and the calf of the subject, with programmable pressure levels and timing. In the present study, all bladders were inflated simultaneously to a suprasystolic pressure of 150 mmHg, with inflation and deflation times of  $\sim 3$  s each. Two

digital pulses are generated by the device to signal the starting time of both inflation and deflation.

**Near-infrared spectroscopy.** Local hemodynamic changes induced by leg compression were measured using a continuous-wave NIRS device (NIRO-200NX; Hamamatsu Photonics, Hamamatsu, Japan), which, besides the classical modified Lambert-Beer method, supports spatially resolved spectroscopy (SRS; 16, 52). Since mechanosensitive vascular reactivity appears to be more prominently expressed by muscular than by cutaneous tissues (57), we focused our attention on SRS parameters, which, being less affected by cutaneous circulation, provide more specific monitoring of muscle tissue (2, 36, 37). Since NIRS cannot discriminate between hemoglobin (Hb) and myoglobin (Mb), all measurements always refer to Hb + Mb in the sample volume (51). In particular, tissue oxygenation index (TOI) indicates the ratio  $(MbO_2 + HbO_2)/(Mb_{tot} + Hb_{tot})$  expressed in percentage (where  $MbO_2$  and  $HbO_2$  refer to oxygenated Mb and Hb, respectively, and  $Mb_{tot}$  and  $Hb_{tot}$  refer to total Mb and Hb, respectively), and tissue hemoglobin index (THI) indicates the concentration of (Hb + Mb) in arbitrary units and is therefore an indicator of blood volume changes. Classical Lambert-Beer parameters [ $O_2Hb$  and  $HHb$ , detecting changes in the concentration of oxygenated and deoxygenated (Hb + Mb), respectively] are only displayed in Fig. 1 and not further considered in the study.

**Hemodynamic measurements.** Measurements of blood velocity in the femoral artery and vein were performed simultaneously using two ultrasound systems (MyLab 25 XVision and MyLab 25 Gold; Esaote, Genoa, Italy) equipped with linear arrays (LA 523; Esaote). Superficial femoral artery and femoral vein were insonated distally to the inguinal ligament. Since these instruments could not measure blood velocity and vessel diameter simultaneously, the latter was measured at the beginning and at the end of every stimulation protocol. Doppler measurements were performed by extending the sample volume over the whole vessel size, echographically displayed (transversal approach) in real time. All blood velocity measurements in the femoral artery were obtained with an insonation angle of  $\sim 60^\circ$  (operating frequency of 6.6 MHz); a higher angle of  $\sim 70^\circ$  (operating frequency of 5 MHz) was used instead to avoid saturation of the recording when assessing the high-speed venous outflow propelled by leg compression. The two probes were placed a few centimeters apart with the ultrasound beam of the proximal probe oriented proximally and that of the distal probe oriented distally to avoid interference between the measurements.

**Experimental setup.** A schematic representation of the experimental setup is reported in Fig. 1A. All experiments were performed in a quiet room with a constant ambient temperature of  $\sim 22$ – $23^\circ C$ . The subject sat upright on an adjustable chair with the back supported by a back rest.

The NIRS probe was located on the lateral head of the gastrocnemius muscle of the right leg (interoptode distance, 4 cm). The IPC device was wrapped around the lower leg, over the NIRS probe. The two echographic probes were maintained in place by dedicated holders for the whole duration of the protocol.

**Experimental protocol.** After 15 min of rest, an initial series of three compressive stimuli with interstimulus interval (ISI) of 160 s was delivered to the subject. After another 4 min of rest, four series of six compressive stimuli were delivered at different frequencies (ISI = 20, 40, 60, and 80 s) in randomized order and separated by 4-min resting intervals. Femoral artery and femoral vein diameters were collected at the beginning and at the end of every stimulation protocol. Diameters were measured along a single direction, since both vessels present a circular cross section in these experimental conditions. The average diameter of the artery was calculated as  $(D_s + 2 \times D_d)/3$ ,  $D_s$  being the systolic and  $D_d$  the diastolic diameter.

**Data acquisition and processing.** The NIRS signals were digitally acquired along with both Doppler audio signals and the digital synchronism signal from the IPC device by a single acquisition system (CED Micro 1041; Cambridge Electronic Design, Cambridge,

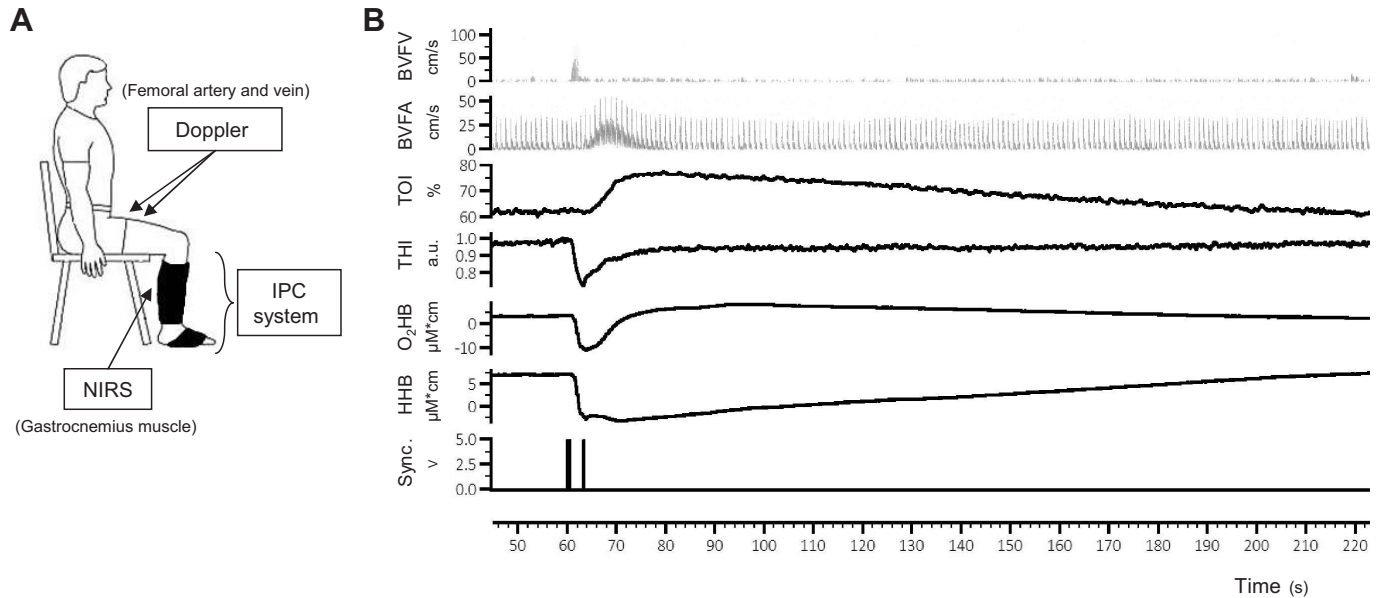


Fig. 1. Experimental setup and typical hemodynamic response to a compressive stimulus. *A*: the experimental setup includes the intermittent pneumatic compression (IPC) system for the compression of the lower limb, eco-Doppler monitoring of blood flow from the femoral vein and femoral artery, and near-infrared spectroscopy (NIRS) monitoring at the lateral head of gastrocnemius muscle. *B*: typical response to leg compression in a representative subject. From top to bottom: blood velocity in the femoral vein (BVFV); blood velocity in the femoral artery (BVFA); tissue oxygenation index (TOI); total hemoglobin index (THI; a.u., arbitrary units); changes in oxygenated hemoglobin ( $O_2Hb$ ) and in deoxygenated hemoglobin (HHb); and synchronism signal (Sync.), the thick and thin bars indicating start of inflation and deflation of the cuff, respectively.

United Kingdom) and stored on the computer for later analysis with Spike2 software (version 6.10; Cambridge Electronic Design).

A specific algorithm was implemented in the Spike2 script language to calculate blood velocity from Doppler audio signals (11, 25). Briefly, power spectra of the audio signals were computed by fast Fourier transform over nonoverlapping epochs lasting 25.6 ms. From each spectrum the maximum frequency of the signal (corresponding to maximum blood velocity) was estimated according to D'Alessio (11), and then the mean frequency was calculated as the average of all frequencies below the maximum, weighted according to spectral amplitude (25). The mean frequency was then time averaged over each cardiac cycle and converted into blood velocity (BV) as follows:  $BV = (MF \times C) / (2F \times \cos \vartheta)$ , where MF is the mean frequency calculated from Doppler shift,  $C$  is the average speed of ultrasound in soft tissue (1,540 m/s),  $F$  is the operating frequency of the Doppler, and  $\vartheta$  is the insonation angle. Blood flow (BF), in milliliters per minute, was then calculated as mean blood velocity times cross-sectional area of the vessel ( $BF = BV \times \pi r^2 \times 60$ , where BV is the blood velocity expressed in cm/s and  $\pi r^2$  is the cross-sectional area of the vessel in  $cm^2$ ).

The response to each compressive stimulus was characterized by the following: precompression arterial blood flow, calculated as the average over the 4 s preceding the compression; precompression TOI; precompression THI; peak arterial blood flow, calculated as the hyperemic peak reached after the compression;  $\Delta TOI$ , calculated as the difference between the peak TOI reached after the compression and precompression TOI; and displaced blood volume, calculated as the product of the area under the curve of the venous blood velocity response and the cross-sectional area of vein.

In addition, the amplitude of the hyperemic response was also calculated as the difference between peak arterial flow and precompression flow.

To assess the extent of attenuation of the response throughout the experimental protocol, changes in blood flow and blood volume were normalized to the changes observed in response to the first delivered compressive stimulus.

**Statistics.** To examine the effect of repetitive leg compression performed at different ISIs on peak blood flow, displaced blood volume, precompression THI, and precompression TOI, a two-way repeated-measures ANOVA was used with factors ISI and repetition (GraphPad Prism v6.0; GraphPad Software, La Jolla, CA). When significance was found, a Dunnett's post hoc test was performed to assess significant changes within each series with respect to the response to the first stimulus. The Pearson coefficient was used to assess the correlation between different variables. All data are expressed as means  $\pm$  SD in the text and means  $\pm$  SE in diagrams. The level of statistical significance was set at  $P < 0.05$ .

## RESULTS

**Single leg compression.** A typical response to a single compressive stimulus is reported in Fig. 1*B*. Venous blood velocity exhibits a prompt and short-lasting increase, peaking  $1.7 \pm 0.2$  s after the beginning and terminating before the end of the compression. The blood volume displaced by compression was on average  $28.3 \pm 14.8$  ml. The increase in arterial blood flow starts immediately after deflation and peaks in  $4.9 \pm 1.4$  s passing from a basal value of  $74.5 \pm 22.7$  ml/min to  $260.2 \pm 83.3$  ml/min during the peak (peak flow is  $3.6 \pm 1.0$  of baseline). Blood flow generally returns within 15–25 s. The response in tissue oxygenation is further delayed. TOI slowly increases (from  $66.4 \pm 5.1$  to  $78.0 \pm 4.0\%$ ) and peaks after  $20.6 \pm 5.1$  s from deflation. Local changes in blood volume are detected by THI exhibiting a rapid decrease during compression followed by a slower return to the basal level, in agreement with the changes in venous and arterial blood flow, respectively.

**Repeated leg compressions.** The hemodynamic response to repetitive leg compression at different ISIs is summarized in Fig. 2, each bar representing the response to a single stimulus.

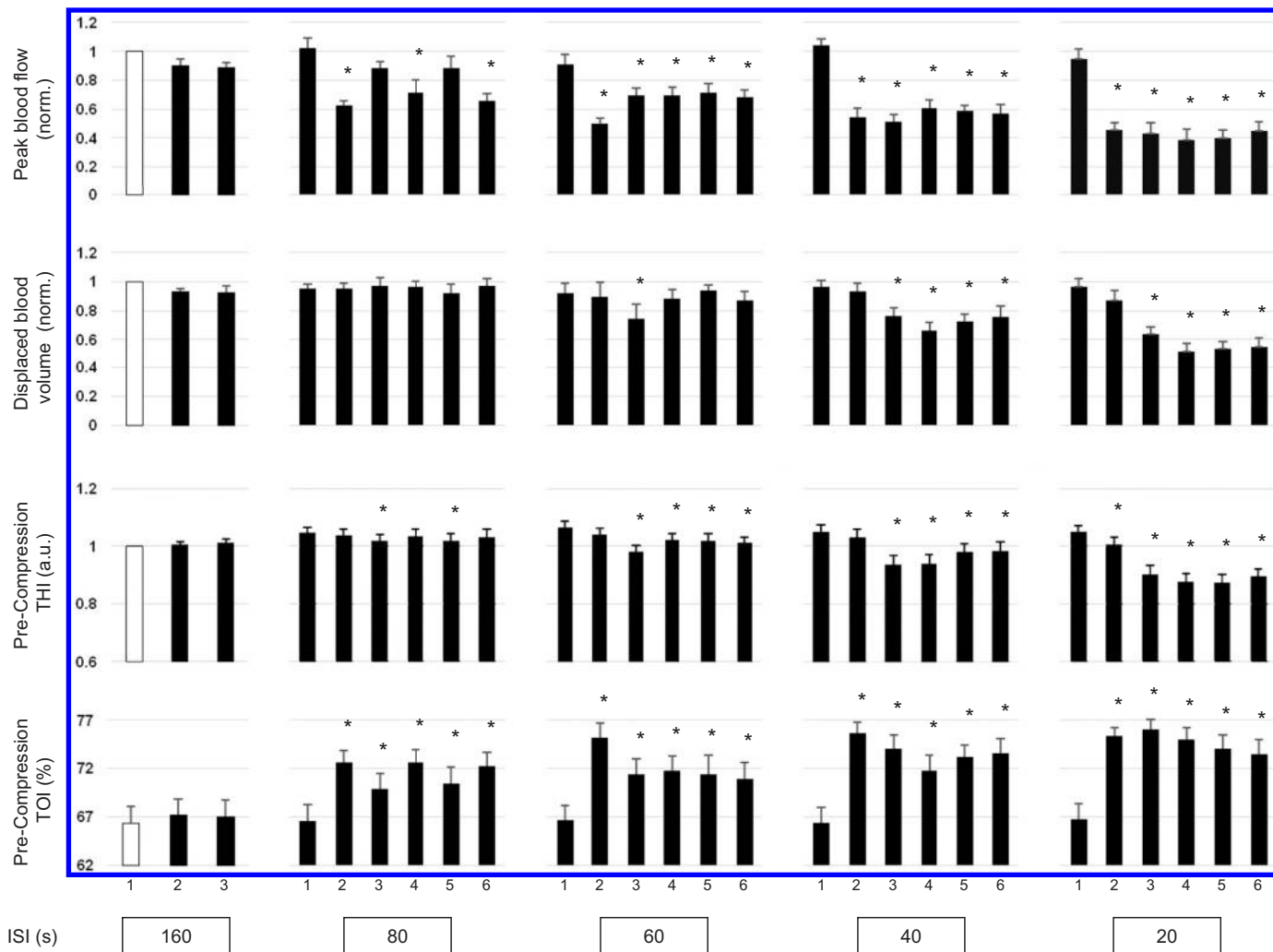


Fig. 2. Hemodynamic responses to repetitive compression at different interstimulus intervals (ISIs). The ISI is indicated at the bottom of each column of bar graphs; each bar refers to the response to a single compressive stimulus. From *top to bottom*: peak (arterial) blood flow, displaced (venous) blood volume, precompression total hemoglobin index (THI; indicating local vascular filling reached before the delivery of the compressive stimulus; a.u., arbitrary units), and precompression tissue oxygenation index (TOI; indicating local tissue oxygenation before the stimulus). For the first three variables and for each subject, responses have been normalized (norm.) to the response to the first stimulus in the 160-s series (white bar). \*Significantly different from the first response in the series ( $P < 0.05$ ).

The upper two rows show the response in terms of peak arterial blood flow and displaced venous blood volume, both variables exhibiting a significant dependence on ISI ( $P < 0.01$ ) and repetition ( $P < 0.01$ ). It can be observed that when ISI = 160 s, the response to subsequent stimuli is unchanged. Unchanged response in terms of peak arterial flow and displaced blood volume is also observed in response to the first compression in each series. However, both parameters exhibit a progressive attenuation although with different time course at ISIs ranging from 20 to 80 s. In particular, hyperemia is consistently reduced starting from the second stimulus in the series, at ISIs ranging from 20 to 60 s, while displaced blood volume is consistently reduced at ISI = 20 and 40 s, starting from the third stimulus. A peculiar pattern is observed at ISI = 80 s, where hyperemia is only attenuated in response to even and not to odd stimuli, while, at the same time, displaced blood volume remains unaffected.

NIRS parameters, shown in the lower rows of Fig. 2, exhibited a significant dependence on repetition ( $P < 0.01$ ),

but not on ISI, along with a significant interaction between the two factors. It can be observed that precompression THI, which can be considered an indicator of vascular filling, qualitatively parallels the changes in displaced blood volume, remaining unchanged at large ISI and exhibiting the most marked reduction at ISI = 20 s. Precompression TOI exhibits, instead, marked increases at all ISIs lower than 160 s starting from the second stimulus in the sequence. It is interesting to observe that its pattern of change is opposite to peak blood flow; that is, hyperemic peak is higher if the precompression TOI is lower. Note also that the oscillating pattern previously observed in peak blood flow at ISI = 80 s is also exhibited by precompression TOI in an opposite way.

To provide a better understanding of the interplay between the different parameters in the peculiar response to repetitive compression at ISI = 80 s, original tracings are reported from a representative subject in Fig. 3. As described in Fig. 1, the first stimulus elicits a marked hyperemia, which results in a marked increase in oxygenation. The following compression,

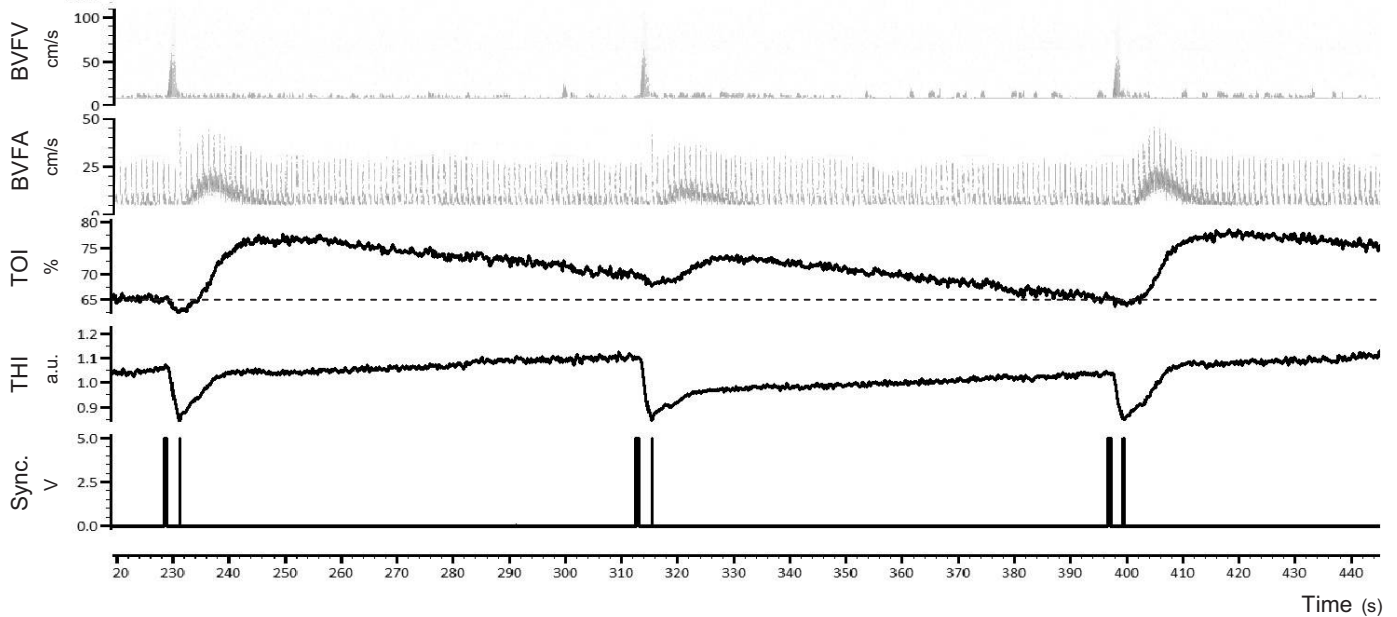


Fig. 3. Original recordings of the response to repetitive leg compression at interstimulus interval of 80 s, from a representative subject. Notations as in Fig. 1. Note the pattern of response of arterial blood velocity in relation to tissue oxygenation. The dashed line represents the initial TOI baseline.

which occurs when the tissue oxygenation is still high, now elicits a much smaller hyperemia, resulting in a proportionally smaller increase in TOI and attenuated vascular refilling in THI. The third compression occurs when the TOI is almost returned to basal levels and the elicited hyperemia resumes its original size. Although it cannot be fully appreciated with this timescale, the venous blood flow response is comparable in all instances as well as the precompression level reached by THI.

Another representative recording illustrating the pattern at  $ISI = 20$  s is reported in Fig. 4. Note the disappearance of the

hyperemic response to the second and subsequent stimuli in spite of the fact that arterial blood flow is returned to basal level. A weak hyperemia reappears only in response to the last stimulus, when also TOI is almost returned to basal level. Note that THI indicates that blood volume is almost fully returned to basal level after the first stimulus (thanks to the marked hyperemia), but not afterward. Accordingly, the venous response is markedly reduced after the third and subsequent stimuli.

In general, a good correlation was found between the peak blood flow during hyperemia and the ensuing increase in

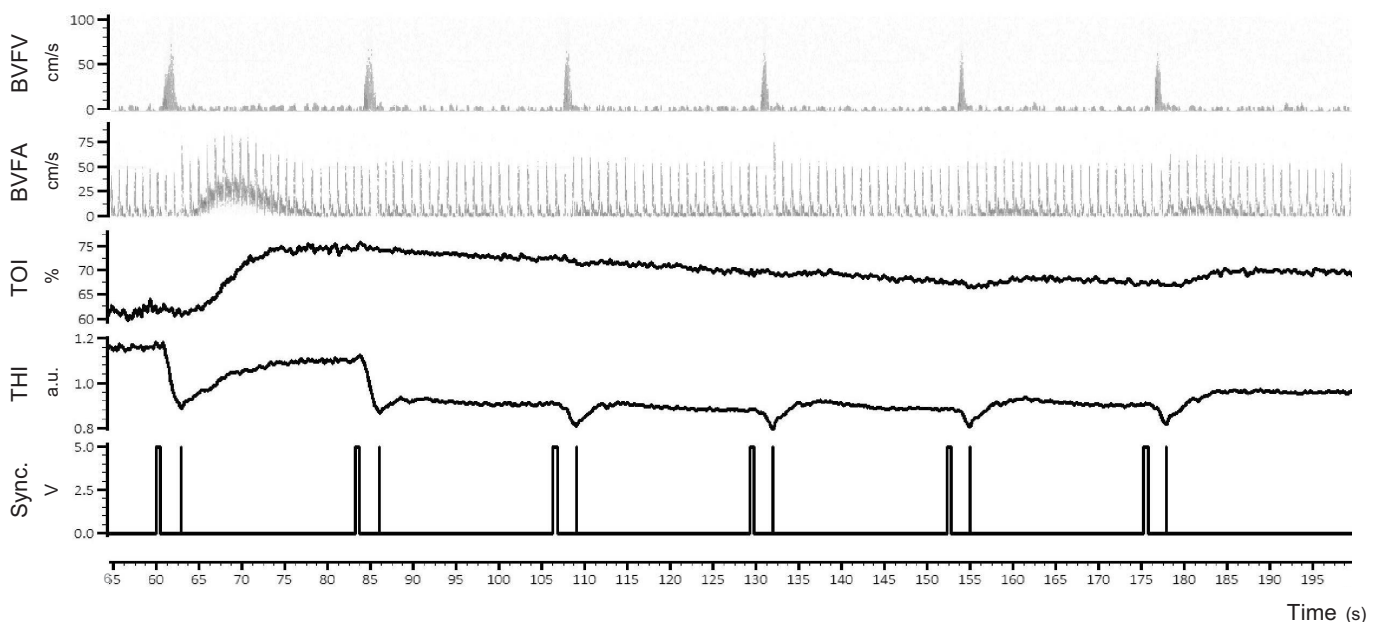


Fig. 4. Original recordings of the response to repetitive leg compression at interstimulus interval of 20 s, from a representative subject. Notations as in Fig. 1. Note the complete disappearance of the hyperemic response (BVFA) after the first compressive stimulation, as long as tissue oxygenation (TOI) remains elevated, and the agreement between the displaced blood volume (area under BVFV) and the current vascular filling (THI).

oxygenation as shown in Fig. 5A, in which all subjects have been pooled and each dot represents the response to a single compression. The overall  $r$  is 0.76 ( $P < 0.05$ ). When individually computed for the different subjects,  $r$  ranged between 0.72 and 0.95 ( $P < 0.05$ ; average  $0.78 \pm 0.1$ ).

In contrast, the hyperemic response was not correlated with the amount of displaced blood volume as shown in Fig. 5B ( $r = 0.34$ , individual  $r$  ranging between  $-0.4$  and  $+0.3$ ).

Figure 5C shows the correlation between precompression TOI and the peak of the hyperemic response, which exhibits an overall  $r = -0.434$  ( $P < 0.05$ ); however, a much higher within-subject correlation is observed:  $-0.78 \pm 0.06$ , individual  $r$  ranging between 0.7 and 0.9 ( $P < 0.05$ ).

In Fig. 5D the amplitude of the hyperemic response (= peak flow - basal flow), instead of peak flow, is plotted vs. pre-compression TOI. Although the general picture resembles that of Fig. 5C, it is here better evidenced that the hyperemia can be almost abolished at high TOI levels. Moreover, the slope of the regression lines,  $m$ , allows us to quantify the dependence of the hyperemic response on tissue oxygenation. On average,  $m = -0.082 \pm 0.026$ , meaning that the compression-induced hyperemia is attenuated by 8% per unitary increase of TOI, with respect to its full amplitude (the one that is evoked in resting conditions).

**Changes in vessel size.** A slight increase in vessel diameter was detected from the comparison of measurements performed at the beginning and at completion of the experimental protocol in both the femoral artery (from  $6.0 \pm 0.8$  to  $6.2 \pm 0.8$  mm,

$P < 0.05$ ) and vein (from  $8.3 \pm 0.9$  to  $8.6 \pm 1.3$  mm,  $P < 0.05$ ).

## DISCUSSION

For the first time a comprehensive approach has been employed for the investigation of rapid compression-induced hyperemia and its adaptation upon repetitive stimulation, which includes continuous assessment of NIRS indicators of changes in local tissue oxygenation and blood volume as well as simultaneous monitoring of arterial inflow and venous outflow. This allowed us to describe the early hyperemic changes taking place at the beginning of IPC treatments at different frequencies and to confirm our initial hypotheses: 1) compression-induced hyperemia elicits proportional increases in local tissue oxygenation; 2) the extent of attenuation of the hyperemic response to subsequent stimuli is related to the current level of tissue oxygenation; and 3) the extent of attenuation is not strictly dependent on the extent of vascular filling and on the ISI, and therefore the attenuation cannot be attributed to the reduced efficacy of the muscle pump or to a simple, time-dependent inactivation mechanism of mechano-sensitive pathways.

**Compression-induced hyperemia increases tissue oxygenation.** A novel observation of the present study is that muscle compression elicits a prominent increase in local tissue oxygenation. This increase is consequent to the induced hyperemia but is much longer lasting. This aspect is important because it

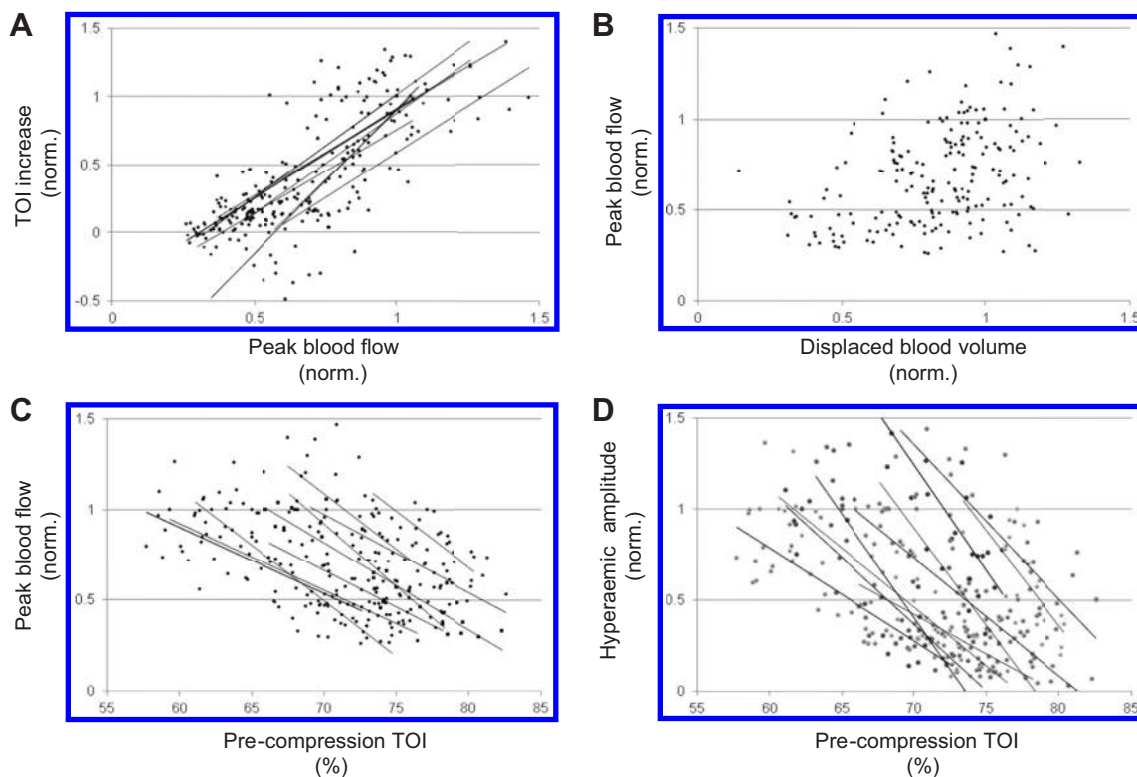


Fig. 5. Scatterplots for assessing the correlation between different variables. Each dot indicates the response to a single compressive stimulus in a single subject. Notations as in Fig. 2;  $n = 10$ . Straight lines indicate linear regressions for individual subjects. Note that the increase in tissue oxygenation is related to the peak blood flow (A); peak blood flow is not related to the displaced blood volume (B) but is inversely related to precompression oxygenation level (C). D: the amplitude of the hyperemic response (peak - baseline) is plotted vs. precompression TOI to indicate that at high oxygenation levels the hyperemic response may be almost completely abolished.

reveals that the return to “control conditions” is not achieved at the end of the hyperemia, which normally occurs within 15–25 s and may instead require up to 100–200 s. This pattern has never been reported for compression-induced hyperemia, but it is in agreement with what occurs in the rapid-onset hyperemia induced by short contractions (53).

It is generally accepted that an increase in perfusion, with unchanged metabolism, increases tissue oxygenation (3, 12). In the present condition, different factors could contribute to the observed TOI increase in response to compression-induced hyperemia: 1) depletion of the venous compartment, which alters the proportion of arterial/venous blood in the sample volume; 2) increased Hb saturation in venous blood due to decreased oxygen extraction, given that the hyperemia occurs in a condition of constant metabolism; and 3) increased saturation of myoglobin. The voiding of the venous compartment does not seem to affect the TOI signal considerably, as no relevant changes are observed immediately after the compression, including those associated with large blood volume changes (see original tracings in Figs. 1, 3, and 4). Unfortunately, NIRS cannot discriminate between Mb and Hb saturation or between arterial and venous compartments; thus no univocal explanation can be provided. Irrespective of the underlying reason, the increase in tissue oxygenation was a very consistent feature of the hemodynamic response to the compression of the resting muscle and exhibited a good correlation with the amplitude of hyperemia (Fig. 5A).

*Is compression-induced hyperemia attenuated by increased tissue oxygenation?* Several lines of evidence from the present study support the finding that elevated tissue oxygenation is the factor responsible for the attenuation of the hyperemia and for the reduced responsiveness to the mechanical stimulus. By looking at the original tracings of Fig. 3 it can be observed that the response to the second compression is smaller compared with the first and the third responses, while TOI is higher than baseline. The same is visible in Fig. 4: the hyperemic response almost disappears during the initial high-oxygenation phase and only later exhibits a tendency to recover, concomitantly with a decrease in TOI. This dependence of peak hyperemia on precompression TOI is also supported by the histograms of Fig. 2 (see opposite patterns of peak blood flow and precompression TOI) and is quantitatively assessed by the correlations in Fig. 5, C and D. Moreover, it appears to be rather linear and rather similar between different subjects. According to these indications, the amplitude of the hyperemic response is attenuated by  $8 \pm 2\%$  per unitary increase of TOI meaning that an increase in TOI by 12.5 points virtually abolishes the response.

Notably, the dependence of the active vessel dilatation on tissue oxygenation may explain why the same short sequence of compressive stimuli elicited opposite effects *in vitro* (7), where tissue hyperoxia does not take place, and *in vivo* (30).

In the several studies investigating hemodynamic effects during IPC treatments this pattern of adaptation of the hyperemia has not been described, possibly because attention was focused on medium- to long-term rather than early effects. Although different devices and patterns of stimulation have been used in previous investigations, an increase in limb perfusion is generally reported, ranging between 20 and 240% and assessed at 5–60 min from the beginning of the treatment (9, 15, 24, 33, 40, 44, 47), which also appears to be little dependent on the stimulation frequency (47). These results are

not readily comparable with the present ones because no steady state was reached in our study. It is reasonable to expect that a certain stable increase in perfusion is obtained with prolonged stimulation, once steady tissue oxygenation is achieved.

*Underlying mechanisms and implications.* As discussed above, the attenuation of the mechanosensitive dilatatory response to multiple compressions could result as a reaction of the tissue to hyperperfusion (generated in response to the first stimulus), which entails the washout of metabolites and alteration of the local milieu, in which  $P_{O_2}$  is the most relevant variable (4, 23, 27). It is well known that low oxygenation stimulates vasodilatation and, conversely, that increased oxygenation leads to vasoconstriction, although the effects generally observed in humans exposed to increased levels of inspired  $P_{O_2}$  are rather small (5, 62). In the latter study, increasing arterial  $P_{O_2}$  from 100 to 2,100 mmHg increased resting vascular conductance only by 20–25% and reduced functional hyperemia by 20% (5). However, it must be observed that tissue  $P_{O_2}$  is differently affected by increased arterial  $P_{O_2}$  and hyperperfusion. In fact, while the hyperbaric hypoxia at 2,100 mmHg increases the amount of oxygen carried to the tissue by ~30% (5), a twofold increase in perfusion results in a 200% increase in oxygen flow. In early studies, reactions to hyperperfusion were investigated on isolated preparations with externally controlled blood supply (21). However, these studies could not provide a clear indication of the time course of the local tissue response, nor could they discriminate between “metabolic” and myogenic response, given that hyperperfusion was produced by increased perfusion pressure, which also resulted in increased transmural pressure (48). In this respect, compression-induced hyperemia offers a peculiar model of (transient) tissue hyperperfusion, characterized by unchanged tissue metabolism, unchanged arterial  $P_{O_2}$ , and most likely unchanged neurohormonal drive.

The prompt counterreaction to compression-induced hyperemia and the concomitant inactivation of the mechanosensitive dilatation upon increased tissue oxygenation fit with the “bang-bang” model of blood flow control, recently proposed by Golub and Pittman (23), according to which the feedback signal ( $O_2^-$ , whose concentration increases in response to increased  $O_2$  availability) carries the information of excessive perfusion and produces vasoconstriction by inactivating the tonically released vasodilators (namely, nitric oxide), the aim of this regulation being to protect the tissue from hyperoxia and prevent excessive perfusion.

Accordingly, vascular mechanosensitivity, which is considered to mediate the rapid dilatation and the anticipatory (feed-forward) hyperemia at the beginning of exercise (8, 30, 43, 60), is promptly abolished if the exercise does not take place, because of the hyperoxygenation produced by hyperperfusion. However, in the case of exercise the hyperoxygenation is quickly reduced even below control levels (18) by increased metabolism, and no limitation to vasodilatation takes place, which results in “functional hyperemia.” The same mechanism is likely to explain why both passive movement hyperemia is attenuated upon repeated stimulation (54, 55) and contraction-induced hyperemia is attenuated after a sequence of muscle compressions (38).

Surprisingly, with one exception (34), no study has ever included NIRS in the characterization of the hyperemic re-



sponse to compression and IPC. Although tissue oxygenation can be considered a major outcome of perfusion, in the short term it does not strictly follow arterial blood flow; for example, in Fig. 2, TOI is maintained at high levels for some time, after the end of hyperemia. On this basis, it might be more appropriate to monitor TOI rather than blood flow to better appreciate the actual effects of the treatment. In addition, adopting NIRS as the monitoring technique gives the possibility to assess the effects specifically on the tissue of interest compared with the more global information provided by blood flow in a large supplying artery.

*Alternative hypothesis 1: vascular refilling and the muscle pump.* The parallelism observed between changes in precompression THI and in displaced blood volume (Fig. 2) suggests that precompression THI is a good indicator of current vascular filling and that its changes mostly reflect volume changes of the venous compartment. By observing its time course after the compressive stimulus we can detect a fast refilling phase, associated with the possible concomitant hyperemia, and a subsequent slow phase, associated with “resting” blood flow. At high ISI, i.e., 80 and 160 s, a complete vascular refill is granted by both a consistent hyperemia and a large time interval. Accordingly, the compressive action of the device displaces a comparable amount of blood volume at every stimulus. At lower ISI, the lack of hyperemia and/or insufficient time for the slow phase to yield a significant contribution may result in incomplete vascular refilling and in a reduction of the blood volume displaced by the subsequent compression. This observation is in agreement with the study by Delis et al., who reported three to four compressions per minute (i.e., ISI = 20 or 15 s) as the optimum stimulation frequency to maintain low venous pressure in the treated limb (13). Valic et al. (61), in the anesthetized dog, estimated a refilling time of <1 s due to the large contraction-induced hyperemia. On the basis of direct foot venous pressure estimation, 2 human studies reported refilling times of 16–40 s after 10 tiptoe movements (42) and pneumatic compression (22). In the present conditions the refill could take place in 10–15 s through the rapid phase in the presence of large hyperemia but could otherwise require >1 min when hyperemia was blunted (Fig. 3).

According to the “muscle pump” effect, an increase in intramuscular pressure empties the venous compartments, producing a decrease in venous pressure, which, in turn, increases the arteriovenous pressure gradient, thus contributing to the ensuing hyperemia. This mechanism is activated both with active muscle contraction as well as with the compression of the passive muscle and has been often considered to explain the larger hyperemic responses observed when compressing (10, 56) or contracting (41, 50) limb muscles below compared with above heart level. However, the issue is still debated (7, 29, 49) because of the conflicting evidence provided by other studies (24, 28, 61). In particular, Jasperse et al. (28) investigated the effect of positional differences on reactive hyperemia, as a model of hyperemia dissociated from the muscle pump. They showed that also reactive hyperemia is larger when evoked below, with respect to above, heart level suggesting that positional effects may be secondary to differences in driving pressure rather than to the muscle pump. The present results support this view through a complementary model, i.e., the muscle pump action dissociated from the hyperemia. This particular condition was observed in several instances such as

the responses to the second compression at ISIs ranging from 20 to 80 s (in Figs. 2 and 4), in which maintained vascular filling and compression-displaced blood volume, i.e., an effective muscle pump, were associated with considerably reduced hyperemia compared with the first compression in the series. This proves that the muscle pump mechanism is not involved in the attenuation of the hyperemia in response to multiple compressions. Whether the muscle pump plays a role in the hyperemic response to the first compressive stimulus cannot be ruled out on the basis of the present data. In fact, from the scatterplot in Fig. 5B we can observe that the largest hyperemic responses were never associated with low displaced blood volume, which suggests that adequate vascular filling may be a necessary condition to express the full response. Investigating the mechanisms behind compression-induced hyperemia was not an aim of this study; further investigations will be necessary to elucidate this issue.

*Alternative hypothesis 2: desensitization of mechanosensitive pathways.* It was previously observed that the hyperemic response to the compressive stimulus progressively reduced to 26% of its original amplitude, with decreasing ISI from 4 min to 2 s (60). On this basis, the hypothesis was put forward that the attenuation could be due to some transient inactivation (desensitization) of mechanosensitive dilatatory mechanisms. This hypothesis was supported by the observation that desensitization upon repeated activation is a characteristic of certain vascular mechanosensitive channels (17, 26). A subsequent human study in which similar stimulation protocols were applied to the forearm qualitatively confirmed the attenuation pattern, although with a less gradual dependence on the ISI (38).

The up-and-down pattern exhibited by compression-induced hyperemia at ISI = 80 s (Figs. 2 and 3) seems to exclude a simple, frequency-dependent desensitization mechanism of mechanosensitive pathways, as previously hypothesized (38, 60). More complex desensitization patterns possibly affecting multiple mechanosensitive pathways cannot be excluded on the basis of the present data. However, to explain the peculiar hyperemic responses observed at ISI = 80 s, such desensitization pattern should exhibit an up-and-down time course, as exhibited by TOI, which would appear to be a quite unlikely coincidence.

*Limitations.* Manual assessment of insonation angles, as required with the transversal approach, is not very accurate and may introduce systematic errors in the calculation of absolute flow values. This is particularly true for assessment of venous blood flow since a wide angle between the vessel axis and the ultrasound beam had to be adopted to avoid saturation of the velocity signal (aliasing). However, the analysis was here focused on relative changes, thereby eliminating errors associated with measurement of the insonation angle.

Diameter of the femoral vein was not continuously monitored. Possible enlargement of the vessel during the passage of the blood volume displaced by the compression may have resulted in underestimation of venous flow.

Diameter of both the femoral artery and vein exhibited a small increase throughout the experimental protocol, which was not accounted for. This may also have led to increasing underestimation of blood flow with time. Since the sequence of the series was randomized, this aspect should not have affected the results.

**Conclusions.** This study demonstrated that the attenuation of hyperemia upon repetitive limb compression is not dependent on vascular filling and the muscle pump or on a simple ISI-dependent desensitization of mechanosensitive structures. In addition, strong evidence is provided supporting the concept that tissue hyperoxygenation is the key signal underlying the inactivation of the rapid dilatatory response to muscle compression. This evidence is, however, indirect, and other studies are necessary to conclusively prove this assertion.

Irrespective of the underlying mechanisms, it is worth emphasizing that the inactivation of the vascular response to the compressive stimulus can be strong enough to abolish hyperemia almost completely, suggesting a role for this phenomenon in protecting tissue from hyperperfusion and oxidative stress.

The hyperemic response to muscle compression is proposed as a peculiar model for the investigation of the response to hyperperfusion characterized by constant arterial  $\text{PO}_2$ , constant tissue metabolism, and modest or absent systemic reactions.

Finally, tissue oxygenation monitoring is recommended to assess the efficacy of IPC treatments, oriented to improve blood perfusion in limbs.

#### ACKNOWLEDGMENTS

We are grateful to the Laboratory of Engineering of Neuromuscular System and Motor Rehabilitation (LISIN, Politecnico di Torino) for lending us the NIRS device. We also thank all the volunteers enrolled in the study.

#### GRANTS

This research was supported by the University of Turin.

#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

#### AUTHOR CONTRIBUTIONS

A.M., W.F., D.M., C.F., and S.R. conceived and designed research; A.M. and G.C. performed experiments; A.M. and G.C. analyzed data; A.M., G.C., W.F., D.M., and S.R. interpreted results of experiments; A.M. and G.C. prepared figures; A.M., G.C., W.F., and D.M. drafted manuscript; C.F. and S.R. edited and revised manuscript; A.M., G.C., W.F., D.M., C.F., and S.R. approved final version of manuscript.

#### REFERENCES

- Barnett A. Using recovery modalities between training sessions in elite athletes: does it help? *Sports Med* 36: 781–796, 2006. doi:10.2165/00007256-200636090-00005.
- Canova D, Roatta S, Bosone D, Micieli G. Inconsistent detection of changes in cerebral blood volume by near infrared spectroscopy in standard clinical tests. *J Appl Physiol* (1985) 110: 1646–1655, 2011. doi:10.1152/jappphysiol.00003.2011.
- Canova D, Roatta S, Micieli G, Bosone D. Cerebral oxygenation and haemodynamic effects induced by nimodipine in healthy subjects. *Funct Neurol* 27: 169–176, 2012.
- Casey DP, Joyner MJ. Local control of skeletal muscle blood flow during exercise: influence of available oxygen. *J Appl Physiol* (1985) 111: 1527–1538, 2011. doi:10.1152/jappphysiol.00895.2011.
- Casey DP, Joyner MJ, Claus PL, Curry TB. Hyperbaric hyperoxia reduces exercising forearm blood flow in humans. *Am J Physiol Heart Circ Physiol* 300: H1892–H1897, 2011. doi:10.1152/ajpheart.00165.2011.
- Clifford PS, Hellsten Y. Vasodilatory mechanisms in contracting skeletal muscle. *J Appl Physiol* (1985) 97: 393–403, 2004. doi:10.1152/jappphysiol.00179.2004.
- Clifford PS, Kluess HA, Hamann JJ, Buckwalter JB, Jasperse JL. Mechanical compression elicits vasodilatation in rat skeletal muscle feed arteries. *J Physiol* 572: 561–567, 2006. doi:10.1113/jphysiol.2005.099507.
- Clifford PS, Tschakovsky ME. Rapid vascular responses to muscle contraction. *Exerc Sport Sci Rev* 36: 25–29, 2008. doi:10.1097/jes.0b013e31815d4ba4.
- Creelius AR, Kirby BS, Luckasen GJ, Larson DG, Dinenna FA. Mechanisms of rapid vasodilation after a brief contraction in human skeletal muscle. *Am J Physiol Heart Circ Physiol* 305: H29–H40, 2013. doi:10.1152/ajpheart.00298.2013.
- Credeur DP, Holwerda SW, Restaino RM, King PM, Crutcher KL, Laughlin MH, Padilla J, Fadel PJ. Characterizing rapid-onset vasodilation to single muscle contractions in the human leg. *J Appl Physiol* (1985) 118: 455–464, 2015. doi:10.1152/jappphysiol.00785.2014.
- D'Alessio T. 'Objective' algorithm for maximum frequency estimation in Doppler spectral analysers. *Med Biol Eng Comput* 23: 63–68, 1985. doi:10.1007/BF02444029.
- Davis SL, Fadel PJ, Cui J, Thomas GD, Crandall CG. Skin blood flow influences near-infrared spectroscopy-derived measurements of tissue oxygenation during heat stress. *J Appl Physiol* (1985) 100: 221–224, 2006. doi:10.1152/jappphysiol.00867.2005.
- Delis KT, Azizi ZA, Stevens RJ, Wolfe JH, Nicolaides AN. Optimum intermittent pneumatic compression stimulus for lower-limb venous emptying. *Eur J Vasc Endovasc Surg* 19: 261–269, 2000. doi:10.1053/ejvs.1999.1047.
- Delis KT, Husmann MJ, Cheshire NJ, Nicolaides AN. Effects of intermittent pneumatic compression of the calf and thigh on arterial calf inflow: a study of normals, claudicants, and grafted arteriopathies. *Surgery* 129: 188–195, 2001. doi:10.1067/msy.2001.110023.
- Delis KT, Labropoulos N, Nicolaides AN, Glenville B, Stansby G. Effect of intermittent pneumatic foot compression on popliteal artery haemodynamics. *Eur J Vasc Endovasc Surg* 19: 270–277, 2000. doi:10.1053/ejvs.1999.1028.
- Delpy DT, Cope M, van der Zee P, Arridge S, Wray S, Wyatt J. Estimation of optical pathlength through tissue from direct time of flight measurement. *Phys Med Biol* 33: 1433–1442, 1988. doi:10.1088/0031-9155/33/12/008.
- Earley S, Brayden JE. Transient receptor potential channels in the vasculature. *Physiol Rev* 95: 645–690, 2015. doi:10.1152/physrev.00026.2014.
- Felici F, Quaresima V, Fattorini L, Sbriccoli P, Filligoi GC, Ferrari M. Biceps brachii myoelectric and oxygenation changes during static and sinusoidal isometric exercises. *J Electromyogr Kinesiol* 19: e1–e11, 2009. doi:10.1016/j.jelekin.2007.07.010.
- Ferraresi C, Maffiolo D, Hajimirzaalian H. A model-based method for the design of intermittent pneumatic compression systems acting on humans. *Proc Inst Mech Eng H* 228: 118–126, 2014. doi:10.1177/0954411913516307.
- Ferraresi C, Maffiolo D, Hajimirzaalian H. Simulation and control of a robotic device for cardio-circulatory rehabilitation. In: *Advances in Robot Design and Intelligent Control*, edited by Rodic A and Borangiu T. Cham, Switzerland: Springer, 2016, p. 357–365. doi:10.1007/978-3-319-21290-6\_36.
- Folkow B. Intravascular pressure as a factor regulating the tone of the small vessels. *Acta Physiol Scand* 17: 289–310, 1949. doi:10.1111/j.1748-1716.1949.tb00576.x.
- Gaskell P, Parrott JC. The effect of a mechanical venous pump on the circulation of the feet in the presence of arterial obstruction. *Surg Gynecol Obstet* 146: 583–592, 1978.
- Golub AS, Pittman RN. Bang-bang model for regulation of local blood flow. *Microcirculation* 20: 455–483, 2013. doi:10.1111/micc.12051.
- González-Alonso J, Mortensen SP, Jeppesen TD, Ali L, Barker H, Damsgaard R, Secher NH, Dawson EA, Dufour SP. Haemodynamic responses to exercise, ATP infusion and thigh compression in humans: insight into the role of muscle mechanisms on cardiovascular function. *J Physiol* 586: 2405–2417, 2008. doi:10.1113/jphysiol.2008.152058.
- Guiot C, Roatta S, Piccoli E, Saccomandi F, Todros T. Quantitative Doppler measures in coiled vessels: investigation on excised umbilical veins. *Ultrasound Med Biol* 25: 1465–1473, 1999. doi:10.1016/S0301-5629(99)00108-8.
- Honoré E, Patel AJ, Chemin J, Suchyna T, Sachs F. Desensitization of mechano-gated K2P channels. *Proc Natl Acad Sci USA* 103: 6859–6864, 2006. doi:10.1073/pnas.0600463103.
- Jackson WF. Arteriolar oxygen reactivity: where is the sensor and what is the mechanism of action? *J Physiol* 594: 5055–5077, 2016. doi:10.1113/JP270192.

28. Jasperse JL, Shoemaker JK, Gray EJ, Clifford PS. Positional differences in reactive hyperemia provide insight into initial phase of exercise hyperemia. *J Appl Physiol* (1985) 119: 569–575, 2015. doi:10.1152/jappphysiol.01253.2013.
29. Joyner MJ, Casey DP. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiol Rev* 95: 549–601, 2015. doi:10.1152/physrev.00035.2013.
30. Kirby BS, Carlson RE, Markwald RR, Voyles WF, Dinunno FA. Mechanical influences on skeletal muscle vascular tone in humans: insight into contraction-induced rapid vasodilatation. *J Physiol* 583: 861–874, 2007. doi:10.1113/jphysiol.2007.131250.
31. Kirby BS, Markwald RR, Smith EG, Dinunno FA. Mechanical effects of muscle contraction do not blunt sympathetic vasoconstriction in humans. *Am J Physiol Heart Circ Physiol* 289: H1610–H1617, 2005. doi:10.1152/ajpheart.00391.2005.
32. Labropoulos N, Leon LR, Jr, Bhatti A, Melton S, Kang SS, Mansour AM, Borge M. Hemodynamic effects of intermittent pneumatic compression in patients with critical limb ischemia. *J Vasc Surg* 42: 710–716, 2005. doi:10.1016/j.jvs.2005.05.051.
33. Labropoulos N, Watson WC, Mansour MA, Kang SS, Littooy FN, Baker WH. Acute effects of intermittent pneumatic compression on popliteal artery blood flow. *Arch Surg* 133: 1072–1075, 1998. doi:10.1001/archsurg.133.10.1072.
34. Manfredini F, Lamberti N, Malagoni AM, Zambon C, Basaglia N, Mascoli F, Manfredini R, Zamboni P. Reliability of the vascular claudication reporting in diabetic patients with peripheral arterial disease: a study with near-infrared spectroscopy. *Angiology* 66: 365–374, 2015. doi:10.1177/0003319714534762.
35. Martin JS, Friedenreich ZD, Borges AR, Roberts MD. Acute effects of peristaltic pneumatic compression on repeated anaerobic exercise performance and blood lactate clearance. *J Strength Cond Res* 29: 2900–2906, 2015. doi:10.1519/JSC.0000000000000928.
36. Messere A, Roatta S. Influence of cutaneous and muscular circulation on spatially resolved versus standard Beer-Lambert near-infrared spectroscopy. *Physiol Rep* 1: e00179, 2013. doi:10.1002/phy2.179.
37. Messere A, Roatta S. Local and remote thermoregulatory changes affect NIRS measurement in forearm muscles. *Eur J Appl Physiol* 115: 2281–2291, 2015. doi:10.1007/s00421-015-3208-7.
38. Messere A, Turturici M, Millo G, Roatta S. Repetitive muscle compression reduces vascular mechano-sensitivity and the hyperemic response to muscle contraction. *J Physiol Pharmacol* 68: 427–437, 2017.
39. Mohrman DE, Sparks HV. Myogenic hyperemia following brief tetanus of canine skeletal muscle. *Am J Physiol* 227: 531–535, 1974.
40. Mortensen SP, Thaning P, Nyberg M, Saltin B, Hellsten Y. Local release of ATP into the arterial inflow and venous drainage of human skeletal muscle: insight from ATP determination with the intravascular microdialysis technique. *J Physiol* 589: 1847–1857, 2011. doi:10.1113/jphysiol.2010.203034.
41. Nädland IH, Walløe L, Toska K. Effect of the leg muscle pump on the rise in muscle perfusion during muscle work in humans. *Eur J Appl Physiol* 105: 829–841, 2009. doi:10.1007/s00421-008-0965-6.
42. Nicolaidis AN, Zukowski AJ. The value of dynamic venous pressure measurements. *World J Surg* 10: 919–924, 1986. doi:10.1007/BF01658640.
43. Poole DC, Ferreira LF, Behnke BJ, Barstow TJ, Jones AM. The final frontier: oxygen flux into muscle at exercise onset. *Exerc Sport Sci Rev* 35: 166–173, 2007. doi:10.1097/jes.0b013e318156e4ac.
44. Roseguini BT, Arce-Esquivel AA, Newcomer SC, Yang HT, Terjung R, Laughlin MH. Intermittent pneumatic leg compressions enhance muscle performance and blood flow in a model of peripheral arterial insufficiency. *J Appl Physiol* (1985) 112: 1556–1563, 2012. doi:10.1152/jappphysiol.01337.2011.
45. Secomb TW. Theoretical models for regulation of blood flow. *Microcirculation* 15: 765–775, 2008. doi:10.1080/10739680802350112.
46. Sheldon RD, Roseguini BT, Laughlin MH, Newcomer SC. New insights into the physiologic basis for intermittent pneumatic limb compression as a therapeutic strategy for peripheral artery disease. *J Vasc Surg* 58: 1688–1696, 2013. doi:10.1016/j.jvs.2013.08.094.
47. Sheldon RD, Roseguini BT, Thyfault JP, Crist BD, Laughlin MH, Newcomer SC. Acute impact of intermittent pneumatic leg compression frequency on limb hemodynamics, vascular function, and skeletal muscle gene expression in humans. *J Appl Physiol* (1985) 112: 2099–2109, 2012. doi:10.1152/jappphysiol.00042.2012.
48. Shepherd JT. Circulation to skeletal muscle. In: *Handbook of Physiology. The Cardiovascular System. Peripheral Circulation and Organ Blood Flow*. Bethesda, MD: Am Physiol Soc, 1983, sect. 2, vol. III, part 1, chapt. 11, p. 319–370.
49. Sheriff D. Point: The muscle pump raises muscle blood flow during locomotion. *J Appl Physiol* (1985) 99: 371–372, 2005. doi:10.1152/jappphysiol.00381.2005.
50. Shoemaker JK, Tschakovsky ME, Hughson RL. Vasodilation contributes to the rapid hyperemia with rhythmic contractions in humans. *Can J Physiol Pharmacol* 76: 418–427, 1998. doi:10.1139/y98-033.
51. Spiers J, Lai N, Zhou H, Sidel GM. Hemoglobin and myoglobin contributions to skeletal muscle oxygenation in response to exercise. *Adv Exp Med Biol* 701: 347–352, 2011. doi:10.1007/978-1-4419-7756-4\_47.
52. Suzuki S, Takasaki S, Ozaki T, Kobayashi Y. Tissue oxygenation monitor using NIR spatially resolved spectroscopy. In: *Proceedings of Optical Tomography and Spectroscopy of Tissue III*. Bellingham, WA: SPIE, 1999, p. 582–592. doi:10.1117/12.356862.
53. Towse TF, Slade JM, Ambrose JA, DeLano MC, Meyer RA. Quantitative analysis of the postcontractile blood-oxygenation-level-dependent (BOLD) effect in skeletal muscle. *J Appl Physiol* (1985) 111: 27–39, 2011. doi:10.1152/jappphysiol.01054.2009.
54. Trinity JD, Groot HJ, Layec G, Rossman MJ, Ives SJ, Runnels S, Gmelch B, Bledsoe A, Richardson RS. Nitric oxide and passive limb movement: a new approach to assess vascular function. *J Physiol* 590: 1413–1425, 2012. doi:10.1113/jphysiol.2011.224741.
55. Trinity JD, McDaniel J, Venturelli M, Fjeldstad AS, Ives SJ, Witman MA, Barrett-O'Keefe Z, Amann M, Wray DW, Richardson RS. Impact of body position on central and peripheral hemodynamic contributions to movement-induced hyperemia: implications for rehabilitative medicine. *Am J Physiol Heart Circ Physiol* 300: H1885–H1891, 2011. doi:10.1152/ajpheart.00038.2011.
56. Tschakovsky ME, Shoemaker JK, Hughson RL. Vasodilation and muscle pump contribution to immediate exercise hyperemia. *Am J Physiol Heart Circ Physiol* 271: H1697–H1701, 1996.
57. Turturici M, Mohammed M, Roatta S. Evidence that the contraction-induced rapid hyperemia in rabbit masseter muscle is based on a mechano-sensitive mechanism, not shared by cutaneous vascular beds. *J Appl Physiol* (1985) 113: 524–531, 2012. doi:10.1152/jappphysiol.00237.2012.
58. Turturici M, Roatta S. Compression-induced hyperaemia in the rabbit masseter muscle: a model to investigate vascular mechano-sensitivity of skeletal muscle. *Physiol Meas* 34: 307–314, 2013. doi:10.1088/0967-3334/34/3/307.
59. Turturici M, Roatta S. Effects of gadolinium chloride on basal flow and compression-induced rapid hyperemia in the rabbit masseter muscle. *J Physiol Pharmacol* 65: 409–415, 2014.
60. Turturici M, Roatta S. Inactivation of mechano-sensitive dilatation upon repetitive mechanical stimulation of the musculo-vascular network in the rabbit. *J Physiol Pharmacol* 64: 299–308, 2013.
61. Valic Z, Buckwalter JB, Clifford PS. Muscle blood flow response to contraction: influence of venous pressure. *J Appl Physiol* (1985) 98: 72–76, 2005. doi:10.1152/jappphysiol.00151.2004.
62. Welch HG, Bonde-Petersen F, Graham T, Klausen K, Secher N. Effects of hyperoxia on leg blood flow and metabolism during exercise. *J Appl Physiol Respir Environ Exerc Physiol* 42: 385–390, 1977.