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

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RESEARCH ARTICLE

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

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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/japplphysiol.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 timescale (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).

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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 P_{O_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 be 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 ± 3.0 yr; weight, 67.9 ± 11.7 kg; height, 176.7 ± 9.7 cm, means ± 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 ~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 ~60° (operating frequency of 6.6 MHz); a higher angle of ~70° (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. 1*A*. All experiments were performed in a quiet room with a constant ambient temperature of ~22–23°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 (Ds + 2 × Dd)/3, Ds being the systolic and Dd 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,

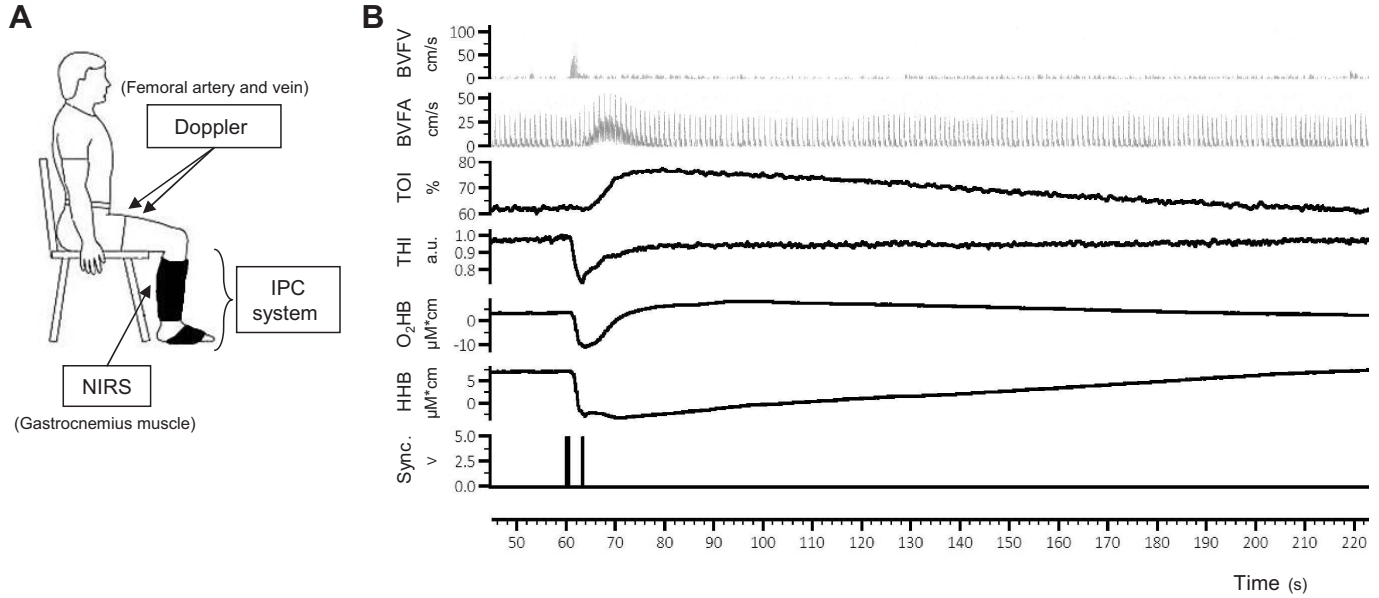

Fig. 1. Experimental setup and typical hemodynamic response to a compressive stimulus. *A*: the experimental setup includes the intermittent pneumatic compression (IPC) system for the compression of the lower limb, eco-Doppler monitoring of blood flow from the femoral vein and femoral artery, and near-infrared 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 ϑ 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 πr^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; ΔTOI, 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 ± SD in the text and means ± 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 ± 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 ± 14.8 ml. The increase in arterial blood flow starts immediately after deflation and peaks in 4.9 ± 1.4 s passing from a basal value of 74.5 ± 22.7 ml/min to 260.2 ± 83.3 ml/min during the peak (peak flow is 3.6 ± 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 ± 5.1 to 78.0 ± 4.0%) and peaks after 20.6 ± 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.

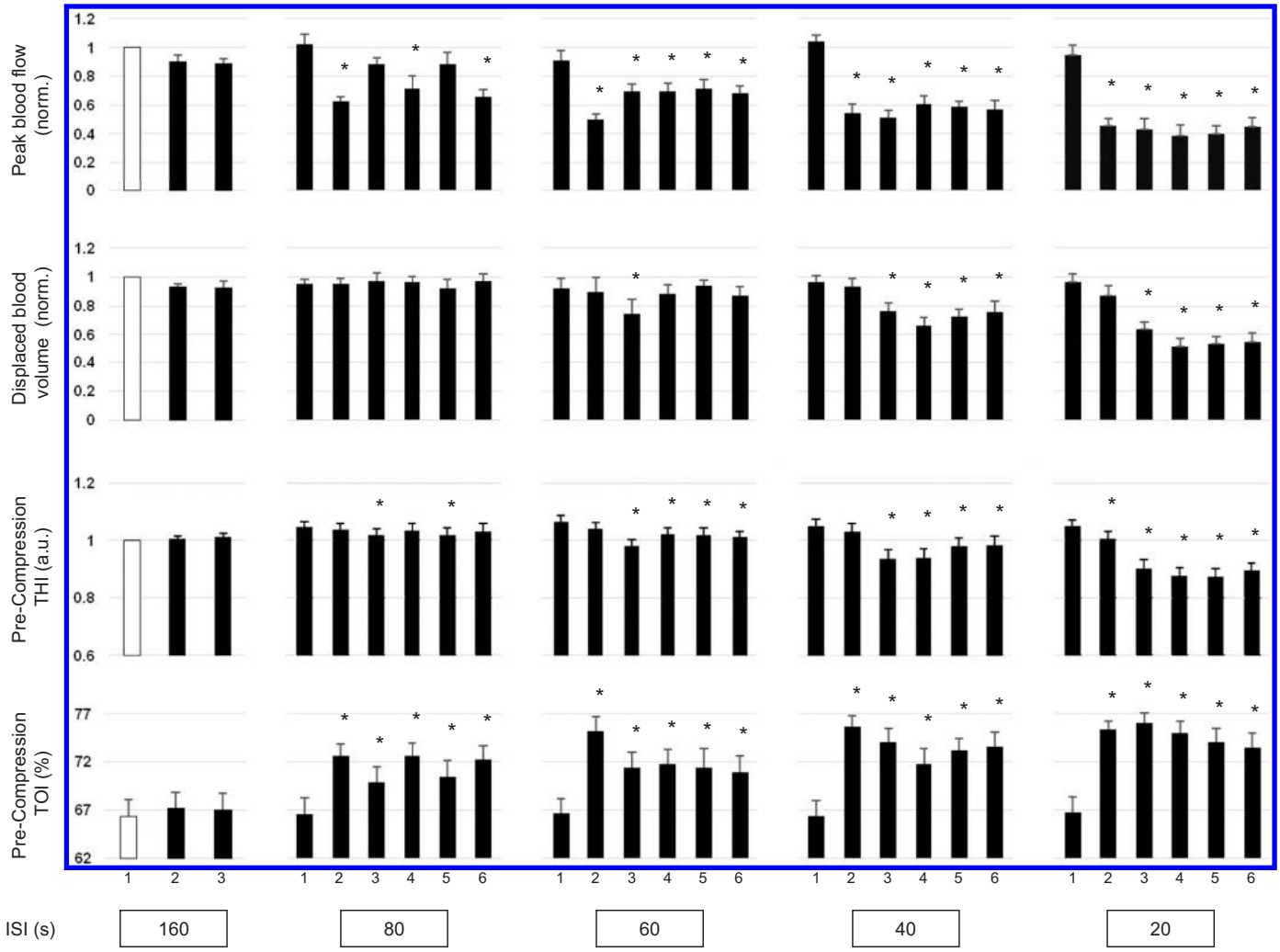

Fig. 2. Hemodynamic responses to repetitive compression at different interstimulus intervals (ISIs). The ISI is indicated at the bottom of each column of bar graphs; each bar refers to the response to a single compressive stimulus. From *top* to *bottom*: peak (arterial) blood flow, displaced (venous) 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,

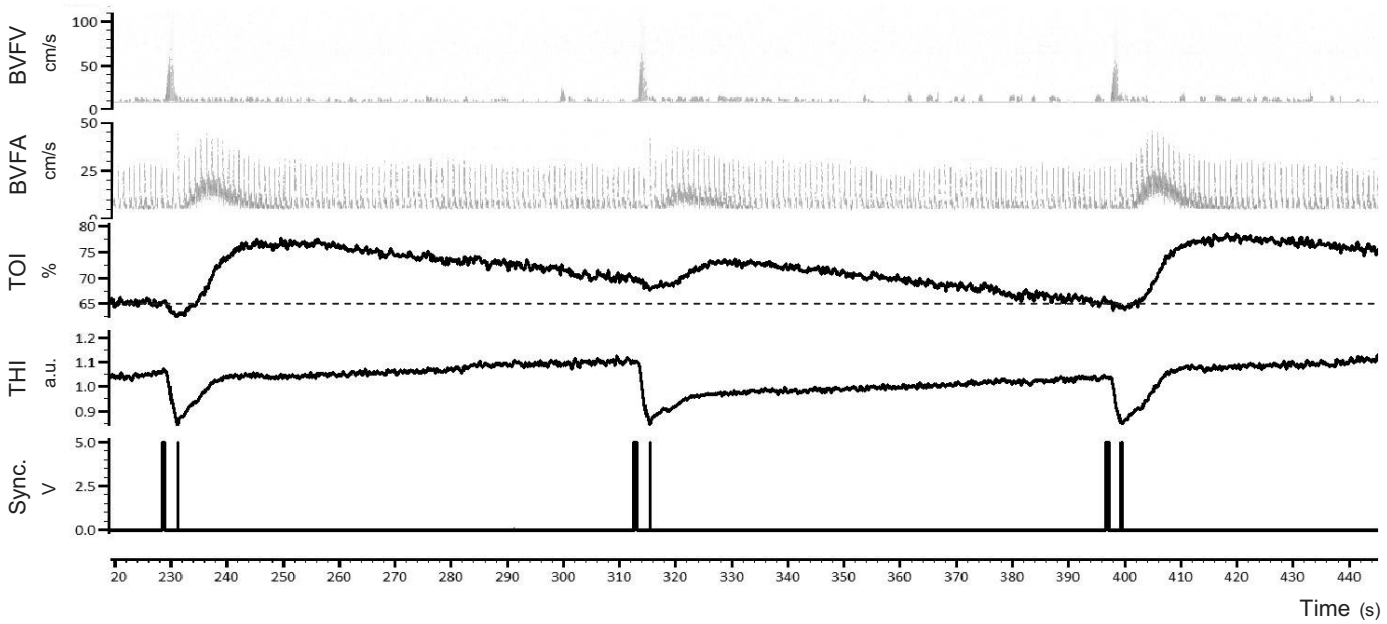

Fig. 3. Original recordings of the response to repetitive leg compression at interstimulus interval of 80 s, from a representative subject. Notations as in Fig. 1. Note the pattern of response of arterial blood velocity in relation to tissue oxygenation. The dashed line represents the initial TOI baseline.

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

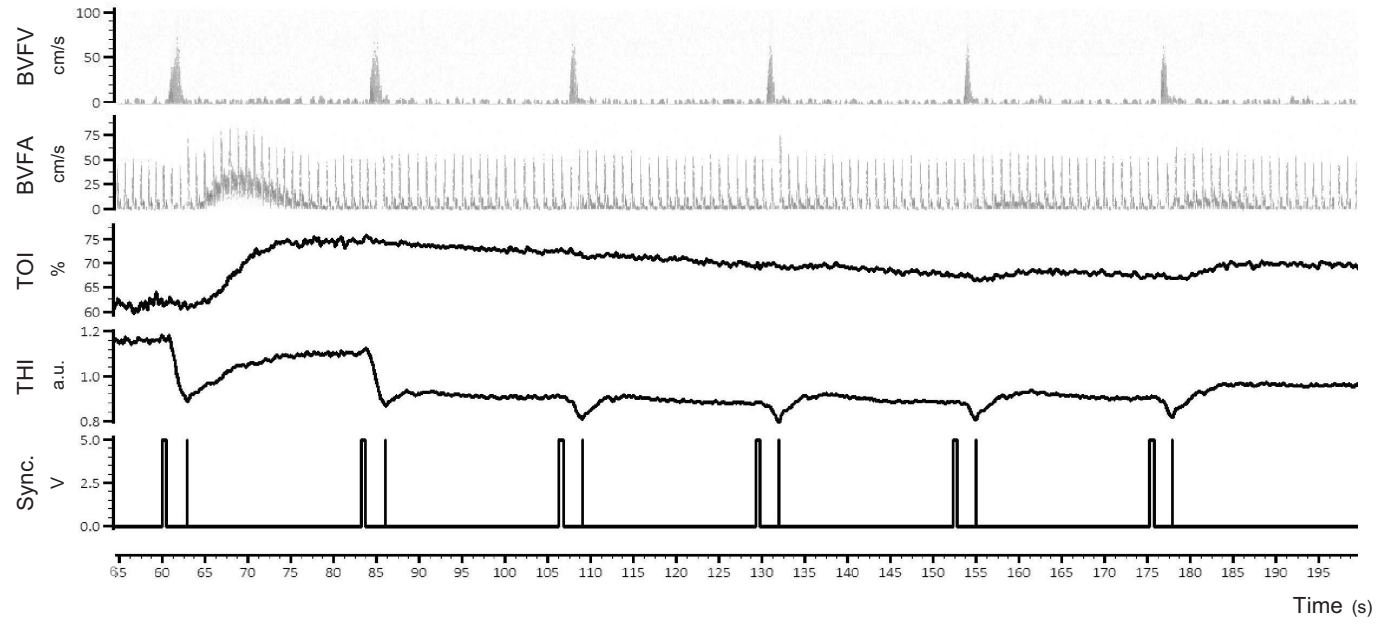

Fig. 4. Original recordings of the response to repetitive leg compression at interstimulus interval of 20 s, from a representative subject. Notations as in Fig. 1. Note the complete disappearance of the hyperemic response (BVFA) after the first compressive stimulation, as long as tissue oxygenation (TOI)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

oxygenation as shown in Fig. 5*A*, 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 ± 0.1).

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

Figure 5*C* 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 ± 0.06, individual r ranging between 0.7 and 0.9 ($P < 0.05$).

In Fig. 5*D* the amplitude of the hyperemic response (= peak flow − basal flow), instead of peak flow, is plotted vs. precompression TOI. Although the general picture resembles that of Fig. 5*C*, 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, $m = -0.082 \pm 0026$, 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 ± 0.8 to 6.2 ± 0.8 mm, $P < 0.05$) and vein (from 8.3 ± 0.9 to 8.6 ± 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 mechanosensitive 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

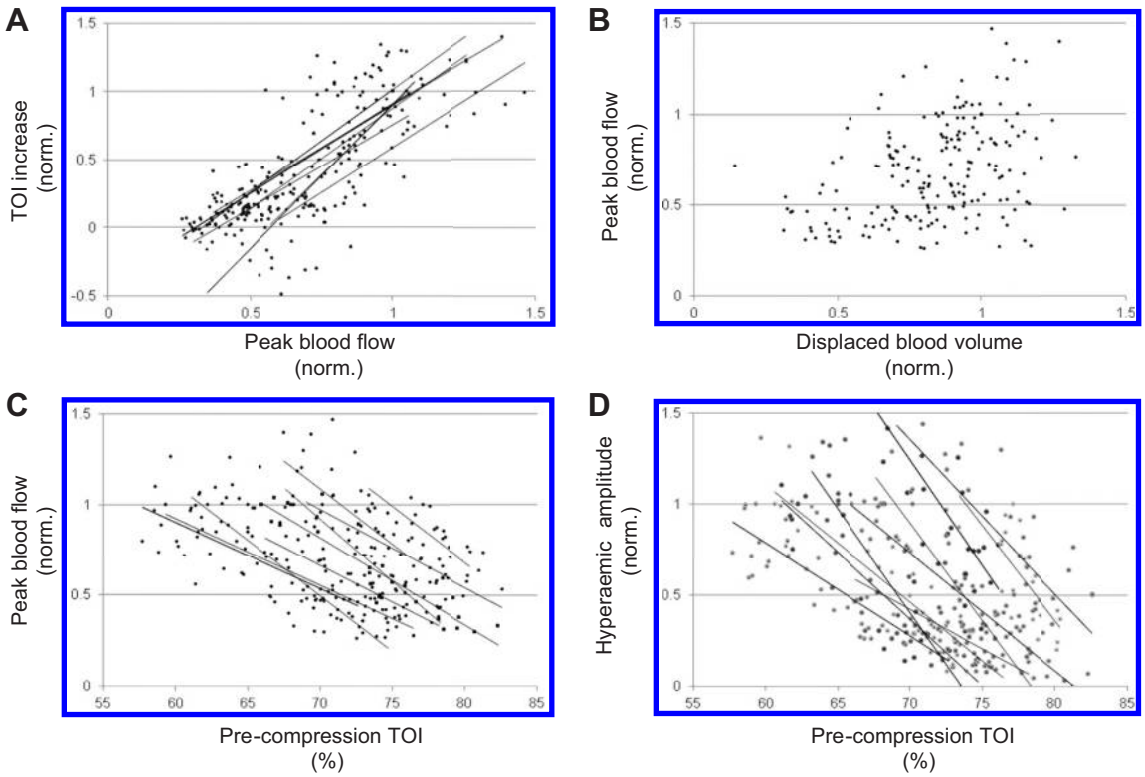

Fig. 5. Scatterplots for assessing the correlation between different variables. Each dot indicates the response to a single compressive stimulus in a single subject. Notations as in Fig. 2; $n = 10$. Straight lines indicate linear regressions for individual subjects. Note that the increase in tissue 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. 5*A*).

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 dilatory 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 (feedforward) 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 vasodilation 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. 5*B* 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 dilatory 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 dilatory 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 P_{O_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.

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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.

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