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

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Running Head Tissue oxygenation modulates compression-induced hyperaemia

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ABSTRACT

Aim

The rapid hyperaemia 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 mechano-sensitive pathways, and reduced efficacy of the muscle pump.

Methods

In 10 healthy subjects short sequences of mechanical compressions (n=3-6; 150 mmHg) of the lower leg were delivered at different inter-stimulus 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, as well as simultaneous echo-Doppler measurement of arterial (superficial femoral artery) and venous (femoral vein) blood flow.

Results

The results indicate that: i) a long lasting (>100 s) increase in local tissue oxygenation follows the compression-induced hyperaemia ; ii) the compression-induced hyperaemia exhibits different patterns of attenuation depending on the inter-stimulus interval; iii) the amplitude of the hyperaemia is not correlated with the amount of blood volume displaced by the compression; iv) the extent of attenuation negatively correlates with tissue oxygenation ($r=-0,78$, $P<0.05$).

Conclusion

Increased tissue oxygenation appears to be the key factor for the attenuation of hyperaemia 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 AND NOTEWORTHY

This study shows that i) the hyperaemia induced by muscle compression produces a long-lasting increase in tissue oxygenation; ii) the hyperaemia produced by subsequent muscle compressions exhibits different pattern of attenuation at different inter-stimulus intervals; iii) the extent of attenuation of the compression-induced hyperaemia 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.

Keywords: muscle blood flow, hyperaemia, muscle compression, tissue oxygenation.

Glossary

IPC intermittent pneumatic compression

ISI inter-stimulus interval

NIRS near-infrared spectroscopy

SRS spatially-resolved spectroscopy

THI total haemoglobin index

TOI tissue oxygenation index

INTRODUCTION

Since the seminal work of Mohrman and Sparks (39) several studies have demonstrated that a rapid and transient hyperaemic 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 5 consecutive compressions was non-significantly attenuated with respect to the response to a single compression. Conversely, Clifford et al (7) using the same pattern of 5 consecutive compressive stimuli on an isolated muscle feed arteries observed a significant increase of the dilatory response as compared to the single compression.

In a recent work Turturici and colleagues 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 hyperaemic 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 hyperaemia 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 hyperaemic effect of intermittent pneumatic compressions (IPC) (14, 15, 32-34), and in experimental studies investigating the mechanisms underlying compression and contraction-induced hyperaemia (9, 24, 31, 40, 44), with the exception of a short report by Tschakowsky et al (56). In this pioneering investigation the authors observed that repetitive compression of the forearm below heart level exhibited a transient hyperaemia 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 hyperaemia 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 the peripheral arterial disease and is pursued in sport medicine for accelerated recovering from fatigue (1, 35). Understanding of the underlying mechanisms is essential for implementing optimal treatments (46). Potential mechanisms underlying attenuation of the hyperaemia during repetitive mechanical stimulation include: 1) inactivation of the mechano-sensitive vasodilatory pathways (60), 2) diminished efficacy of the muscle pump (56), and 3) local regulatory mechanisms that may be activated in response to hyper-perfusion (30, 56). Unfortunately, none of these possibilities is supported by a solid experimental evidence. In particular, 1) mechano-sensitive 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, however, 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 hyper-perfusion 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 a compression-induced hyperaemia could then trigger a constrictor response and limit further hyperaemic events in response to subsequent mechanical stimuli.

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

In order to assess changes in tissue oxygenation, the 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 years; weight: 67.9 ± 11.7 kg; height: 176.7 ± 9.7 cm) were recruited for the present study. All subjects were normotensive and non-obese.

The study conformed to the standards set by the Declaration of Helsinki and was approved by the Local

Ethical Committee (Prot. # 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 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 supra-systolic pressure of 150 mmHg, with inflation and deflation times of about 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 City, Japan), which, besides the classical modified-Lambert-Beer method, supports spatially-resolved spectroscopy (SRS) (16, 52). Since mechano-sensitive vascular reactivity appears to be more prominently expressed by muscular than cutaneous tissues (57) we focused our attention on SRS parameters which, being less affected by cutaneous circulation, provide a more specific monitoring of muscle tissue (2, 36, 37). Since NIRS cannot discriminate between haemoglobin (Hb) and myoglobin (Mb), all measurements always refer to Hb+Mb in the sample volume (51). In particular, TOI (tissue oxygenation index) indicates the ratio (MbO_2+HbO_2)/(Mbtot+Hbtot) expressed in percentage, and THI (tissue haemoglobin index) 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 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, Genoa, Italy). 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 femoral artery were obtained with insonation angle of about 60° (operating frequency of 6.6 MHz) instead, a higher angle of about 70° (operating frequency of 5 MHz) was used in order to avoid saturation of the recording when assessing the high-speed venous outflow propelled by leg compression. The two probes were placed

few centimeters apart with the ultrasound beam of the proximal probe oriented proximally and the one of the distal probe oriented distally, in order to avoid interference between the measurements.

Experimental set-up

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 about 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 gastrocnemius muscle of the right leg (inter-optode 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 3 compressive stimuli with inter-stimulus interval (ISI) of 160 s was delivered to the subject. After other 4 min of rest four series of 6 compressive stimuli were delivered at different frequency (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. 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, UK) and stored on the computer for later analysis with Spike2 software (version 6.10, Cambridge Electronic Design, UK).

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 the Fast Fourier Transform over non-overlapping 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), 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, $BV = (MF * C)/(2F * \cos\vartheta)$, where MF is the mean frequency calculated from Doppler shift, C the averaging speed of ultrasound in soft tissue (1540 m/s), F the operating frequency of the Doppler, and ϑ the insonation angle). Blood flow, in ml/min, was then calculated

as mean blood velocity times cross-sectional area of the vessel ($BF = BV * \pi r^2 * 60$, where BV is the blood velocity expressed in cm/s, and πr^2 the cross sectional area of the vessel in cm^2).

The response to each compressive stimulus was characterized by: pre-compression arterial blood flow, calculated as the average over the 4 s preceding the compression; pre-compression TOI; pre-compression THI; peak arterial blood flow, as the hyperaemic peak reached after the compression; Δ TOI, calculated as the difference between the peak TOI reached after the compression and pre-compression TOI; 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 hyperaemic response was also calculated as the difference between peak arterial flow and pre-compression flow.

In order 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 ISI on peak blood flow, displaced blood volume, pre-compression THI and pre-compression TOI, a two-way repeated-measures ANOVA was used with factors ISI and repetition (GraphPad Prism v 6.0, 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. Pearson's coefficient was used to assess the correlation between different variables. All data are expressed as means ± standard deviation in the text and means ± standard error 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 figure 1B. 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-1 to 260.2 ± 83.3 ml min-1 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 ISI is summarized in Fig 2, each column representing the response to a single stimulus. 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. Instead, both parameters exhibit a progressive attenuation although with different time course at ISI ranging from 20 to 80 s. In particular, the hyperaemia is consistently reduced starting from the second stimulus in the series, at ISI 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 hyperaemia 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 pre-compression 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. Pre-compression 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: i.e., hyperaemic peak is higher if the pre-compression TOI is lower. Note also that the oscillating pattern previously observed in peak blood flow at ISI = 80 s is also exhibited by pre-compression TOI in an opposite way.

In order 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 hyperaemia which results in a marked increase in oxygenation. The following compression, which occurs when the tissue oxygenation is still high, now elicits a much smaller hyperaemia , 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 hyperaemia resumes its original size. Although it cannot be fully appreciated with this time scale, the venous blood flow response is comparable in all instances as well as the pre-compression level reached by THI.

Another representative recording illustrating the pattern at ISI = 20 s is reported in fig 4. Note the disappearance of the hyperaemic response to the second and subsequent stimuli in spite of the fact that arterial blood flow is returned to basal level. A weak hyperaemia 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 hyperaemia) but not afterwards. 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 hyperaemia and the ensuing increase in 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).

On the contrary the hyperaemic 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).

Fig 5C shows the correlation between pre-compression TOI and the peak of the hyperaemic response which is exhibiting 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. 5D the amplitude of the hyperaemic response (= peak flow-basal flow) instead of peak flow is plot vs. pre-compression TOI. While the general pictures resembles that of Fig. 5C, it is here better evidenced that the hyperaemia can be almost abolished at high TOI levels. Moreover, the slope of the regression lines, *m*, allows to quantify the dependence of the hyperaemic response on tissue oxygenation. On average, m = -0.082 ± 0026 meaning that the compression-induced hyperaemia 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 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 the rapid compression-induced hyperaemia 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 hyperaemic changes taking place at the beginning of IPC treatments at different frequencies, and to confirm our initial hypotheses: i) the compression-induced hyperaemia elicits proportional increases in local tissue oxygenation; ii) the extent of attenuation of the hyperaemic response to subsequent stimuli is related to the current level of tissue oxygenation; iii) the extent of attenuation is not strictly dependent on the extent of vascular filling and on the ISI, 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.

301

302 *Compression-induced hyperaemia increases tissue oxygenation*

303 A novel observation of the present study is that muscle compression elicits a prominent increase in local
304 tissue oxygenation. This increase is consequent to the induced hyperaemia but is much longer lasting. This
305 aspect is important because it reveals that the return to "control conditions" is not achieved at the end of the
306 hyperaemia, which normally occurs within 15-25 s and may instead require up to 100 - 200 s. This pattern
307 has never been reported for compression-induced hyperaemia but it is in agreement with what occurs in the
308 rapid-onset hyperaemia induced by short contractions (53).

309 It is generally accepted that an increase in perfusion, with unchanged metabolism, increases tissue
310 oxygenation (3, 12). In the present condition, different factors could contribute to the observed TOI increase
311 in response to compression-induced hyperaemia: 1) depletion of the venous-compartment, which alters the
312 proportion of arterial/venous blood in the sample volume; 2) increased Hb saturation in venous blood due to
313 decreased oxygen extraction, given that the hyperaemia occurs in a condition of constant metabolism; 3)
314 increased saturation of myoglobin. The voiding of venous compartment does not seem to affect the TOI
315 signal considerably, as no relevant changes are observed immediately after the compression, including those
316 associated with large blood volume changes (see original tracings in Figs. 1, 3 and 4). Unfortunately, NIRS
317 cannot discriminate between Mb and Hb saturation nor between arterial and venous compartments, thus no
318 univocal explanation can be provided. Irrespective of the underlying reason, the increase in tissue
319 oxygenation was a very consistent feature of the hemodynamic response to the compression of the resting
320 muscle and exhibited a good correlation with the amplitude of hyperaemia (Fig. 5A).

321

322 *Is compression-induced hyperaemia attenuated by increased tissue oxygenation?*

323 Several lines of evidence from the present study support the finding that elevated tissue oxygenation is the
324 factor responsible for the attenuation of the hyperaemia and for the reduced responsiveness to the mechanical
325 stimulus. By looking at the original tracings of Fig. 3 it can be observed that the response to the second
326 compression is smaller as compared to the first and the third responses, while TOI is higher than baseline.
327 The same is visible in Fig.4: the hyperaemic response almost disappears during the initial high oxygenation
328 phase and only later exhibits a tendency to recover, concomitantly with a decrease in TOI. This dependence
329 of peak hyperaemia on pre-compression TOI is also supported by the histograms of Fig. 2 (see opposite
330 patterns of peak blood flow and pre-compression TOI) and is quantitatively assessed by the correlations in
331 Fig. 5 C and D. Moreover, it appears to be rather linear and rather similar between different subjects.
332 According to these indications, the amplitude of the hyperaemic response is attenuated by 8 ± 2 % per
333 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 hyperaemia has not been described, possibly because the attention was focused on medium-long term rather than on 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 being assessed at 5-60 min from the beginning of the treatment (9, 15, 24, 33, 40, 44, 47), which also appear 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 mechano-sensitive dilatory response to multiple compressions could result as a reaction of the tissue to the hyper-perfusion (generated in response to the first stimulus), which entails the washout of metabolites and alteration of the local milieu in which PO_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 PO_2 are rather small (5, 62). In the latter study, increasing arterial PO_2 from 100 to 2100 mmHg increased resting vascular conductance only by 20-25% and reduced functional hyperaemia by 20% (5). However it must be observed that tissue PO_2 is differently affected by increased arterial PO_2 and hyper-perfusion. In fact while the hyperbaric hypoxia at 2100 mmHg increases the amount of oxygen carried to the tissue by about 30% (5) a 2-fold increase in perfusion results in a 200% increase in oxygen flow. In early studies reactions to hyper-perfusion 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 hyper-perfusion was produced by increased perfusion pressure which also resulted in increased transmural pressure (48). In this respect, the compression-induced hyperaemia offers a peculiar model of (transient) tissue hyper-perfusion, characterized by unchanged tissue metabolism, unchanged arterial PO_2 and most likely unchanged neuro-hormonal drive.

The prompt counter-reaction to the compression-induced hyperaemia and the concomitant inactivation of the mechano-sensitive dilatation upon increased tissue oxygenation fits with the “bang-bang” model of blood flow control, recently proposed by Golub & 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 operates a vasoconstriction by inactivating the tonically released vasodilators (namely, nitric oxide), aim of this regulation being to protect the tissue from hyperoxia and prevent excessive perfusion.

Accordingly, the vascular mechano-sensitivity, which is considered to mediate the rapid dilatation and the anticipatory (feed-forward) hyperaemia at the beginning of exercise (8, 30, 43, 60) is promptly abolished if the exercise does not take place, due to the hyper-oxygenation produced by the hyper-perfusion. Instead, in the case of exercise the hyper-oxygenation is quickly reduced even below control levels (18) by increased metabolism and no limitation to vasodilation takes place, which results in the "functional hyperaemia". The same mechanism is likely to explain why both passive movement hyperaemia is attenuated upon repeated stimulation (54, 55) and contraction-induced hyperaemia 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 hyperaemic response 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, e.g., in Fig. 2 TOI is maintained at high levels for some time, after the end of hyperaemia. On this basis, it might be more appropriate to monitor TOI rather than blood flow in order 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, as compared to the more global information provided by blood flow in an large supplying artery.

Alternative hypothesis 1: Vascular refilling and the muscle pump

The parallelism observed between changes in pre-compression THI and in displaced blood volume (Fig.2), suggests that pre-compression 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 to the possible concomitant hyperaemia, and a subsequent slow phase, associated to “resting” blood flow. At high ISI, i.e., 80 and 160 s, a complete vascular refill is granted by both a consistent hyperaemia and a large time interval. Accordingly, the compressive action of the device displaces comparable amount of blood volume at every stimulus. At lower ISI, the lack of hyperaemia 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 and colleagues who reported 3 to 4 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 less than 1 s due to the large contraction-induced hyperaemia. Based on direct foot venous pressure estimation, two human studies reported refilling times of 16 - 40 s after 10 tip–toe 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 hyperaemia but could otherwise require more than one minute when hyperaemia 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 artero-venous pressure gradient thus contributing to the ensuing hyperaemia. 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 hyperaemic responses observed when compressing (10, 56) or contracting (41, 50) limbs muscles below as compared to above heart level. However the issue is still debated (7, 29, 49) due to the conflicting evidence provided by other studies (24, 28, 61). In particular, Jasperse et al. investigated the effect of positional differences on reactive hyperaemia, as a model of hyperaemia dissociated from the muscle pump. They showed that also reactive hyperaemia 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 hyperaemia. This particular condition was observed in several instances such as the responses to the second compression at ISI ranging from 20 to 80 s (in Fig 2 and in Fig 4), in which maintained vascular filling and compression-displaced blood volume, i.e., an effective muscle pump, was associated with a considerably reduced hyperaemia, as compared to the first compression in the series. This proves that the muscle pump mechanism is not involved in the attenuation of the hyperaemia in response to multiple compressions. Whether the muscle pump plays a role in the hyperaemic response to the first compressive stimulus cannot be ruled out based on the present data. In fact, from scatter plot in Fig 5B we can observe that the largest hyperaemic 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 hyperaemia was not an aim of this study; further investigations will be necessary to elucidate this issue.

Alternative hypothesis 2: Desensitization of mechano-sensitive pathways

It was previously observed that the hyperaemic 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 mechano-sensitive dilatory mechanisms. This hypothesis was supported by the observation that desensitization upon repeated activation is a characteristic of certain vascular mechano-sensitive 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 hyperaemia at ISI = 80 s (Fig. 2 and Fig. 3) seems to exclude a simple, frequency-dependent, desensitization mechanism of mechano-sensitive pathways, as previously hypothesized (38, 60). More complex desensitization patterns possibly affecting multiple

mechanosensitive pathways cannot be excluded based on the present data. However, in order to explain the peculiar hyperaemic responses observed at ISI=80 s, such desensitization pattern should exhibit an up-and-down time course, as exhibited by TOI, which would appear 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 in order 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 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 hyperaemia upon repetitive limb compression is not dependent on vascular filling and the muscle pump nor on a simple ISI-dependent desensitization of mechano-sensitive structures. In addition, strong evidence is provided, supporting the concept that tissue hyper-oxygenation 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 the hyperemia almost completely, suggesting a role for this phenomenon in protecting the tissue form hyperperfusion and oxidative stress.

The hyperaemic response to muscle compression is proposed as a peculiar model for the investigation of the response to hyper-perfusion characterized by constant arterial pO_2, constant tissue metabolism as well as 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.

Acknowledgements

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 Torino.

Disclosures

No competing interest to declare.

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LEGENDS TO FIGURES

Fig 1

Experimental setup and typical hemodynamic response to a compressive stimulus.

A) The experimental setup includes: the IPC system for the compression of the lower limb, eco-Doppler monitoring of blood flow from femoral vein and femoral artery, and NIRS monitoring at lateral head of gastrocnemius muscle. B) Typical response to leg compression in a representative subject. From top to bottom: blood velocity in femoral vein (BVFV), blood velocity in femoral artery (BVFA), tissue oxygenation index (TOI), total hemoglobin index (THI), changes in oxygenated hemoglobin (O_2Hb) and in deoxygenated hemoglobin (HHb) and the synchronism signal (Sync.), the thick and thin bars indicating start of inflation and deflation of the cuff, respectively.

Fig 2

Hemodynamic responses to repetitive compression at different inter-stimulus intervals (ISI).

The ISI is indicated at the bottom of each column of bar- diagrams; each bar refers to the response to a single compressive stimulus. From top to bottom: Peak (arterial) blood flow, displaced (venous) blood volume, Pre-compression THI (indicating local vascular filling reached before the delivery of the compressive stimulus); Pre-compression TOI (indicating local tissue oxygenation before the stimulus). For the first three variables

641 and for each subject, responses have been normalized to the response to the first stimulus in the 160-s series
642 (white bar). * significantly different from the first response in the series ($p<0.05$)

643 **Fig 3**

644 **Original recordings of the response to repetitive leg compression at inter-stimulus interval = 80 s, from**
645 **a representative subject.**

646 Notations as in Fig.1. Note the pattern of response of arterial blood velocity in relation to tissue oxygenation.
647 The dotted line represents the initial TOI baseline.

648 **Fig 4**

649 **Original recordings of the response to repetitive leg compression at inter-stimulus interval = 20 s, from**
650 **a representative subject.**

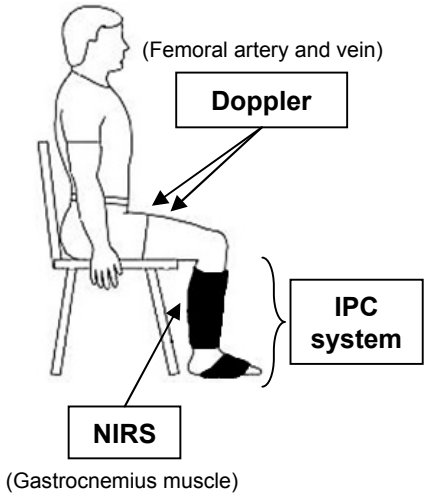
651 Notations as in Fig.1. Note the complete disappearance of the hyperaemic response (BVFA) after the first
652 compressive stimulation, as long as tissue oxygenation (TOI) remains elevated, and the agreement between
653 the displaced blood volume (area under BVFV) and the current vascular filling (THI).

654 **Fig 5**

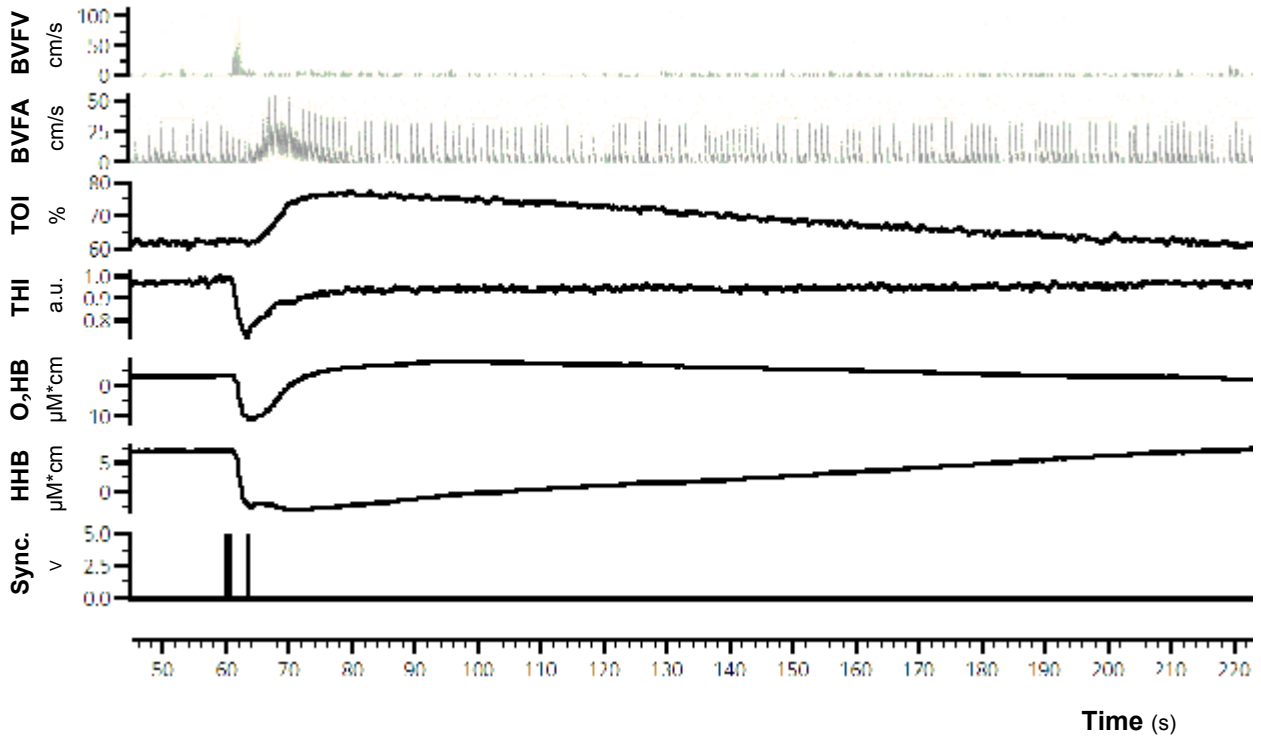
655 **Scatter plots for assessing the correlation between different variables.** Each dot indicates the response to
656 a single compressive stimulus in a single subject. Notations as in Fig. 2. (n=10). Straight lines indicate linear
657 regressions for individual subjects. Note that: the increase in tissue oxygenation is related to the peak blood
658 fow (A); Peak blood flow is not related to the displaced blood volume (B) but is inversely related to pre-
659 compression oxygenation level. In D the amplitude of the hyperaemic response (peak-baseline) is plot vs
660 pre-compression TOI to indicate that at high oxygenation levels the hyperaemic response may be almost
661 completely abolished.

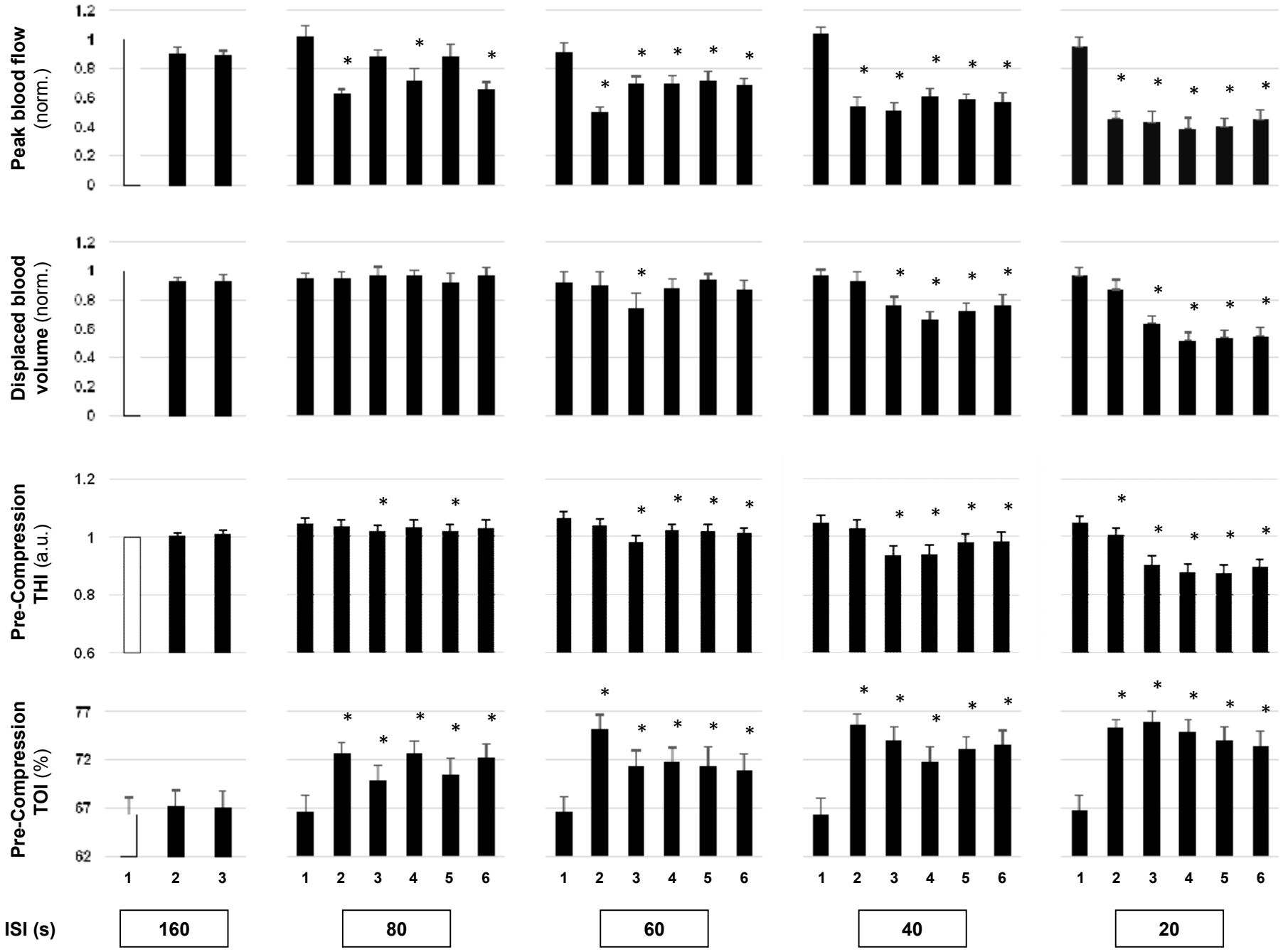
662

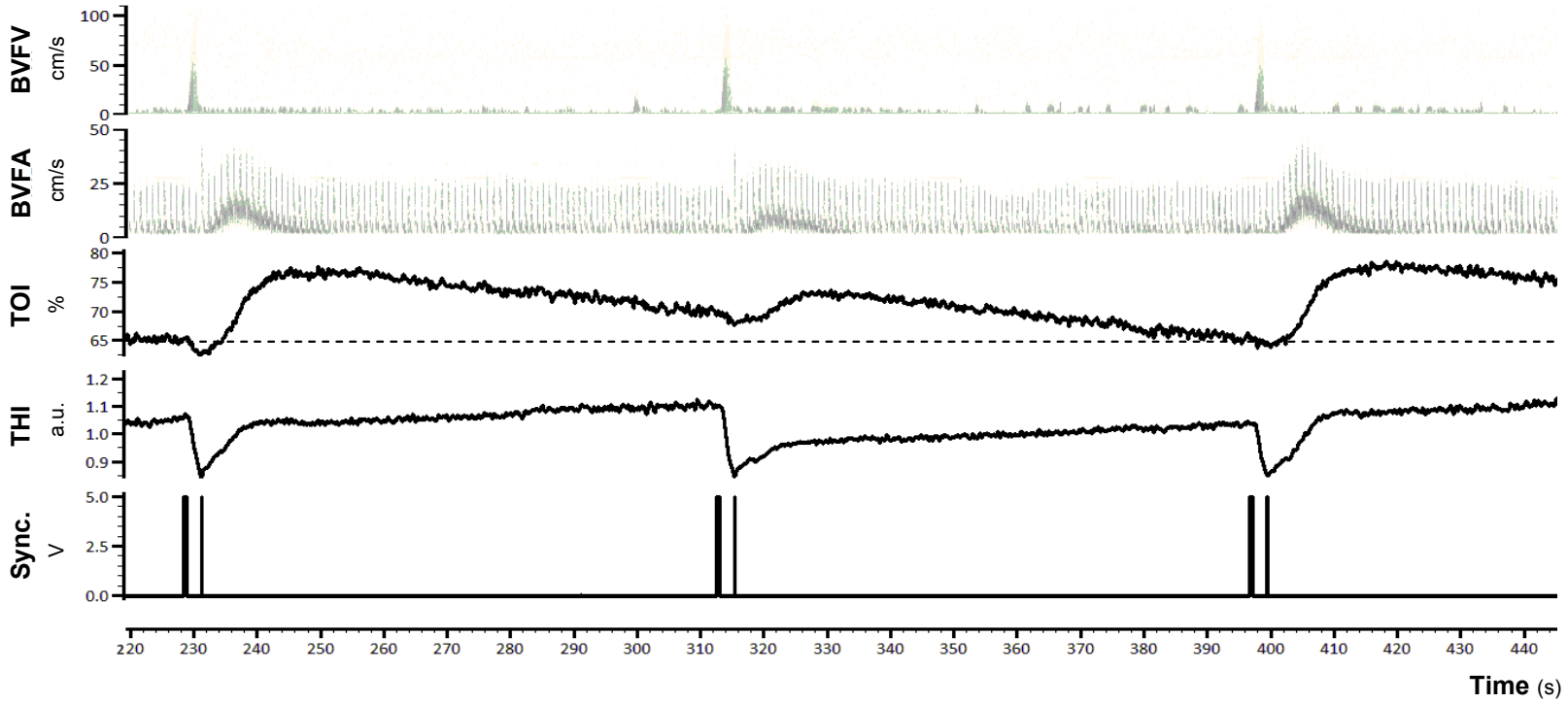
A)

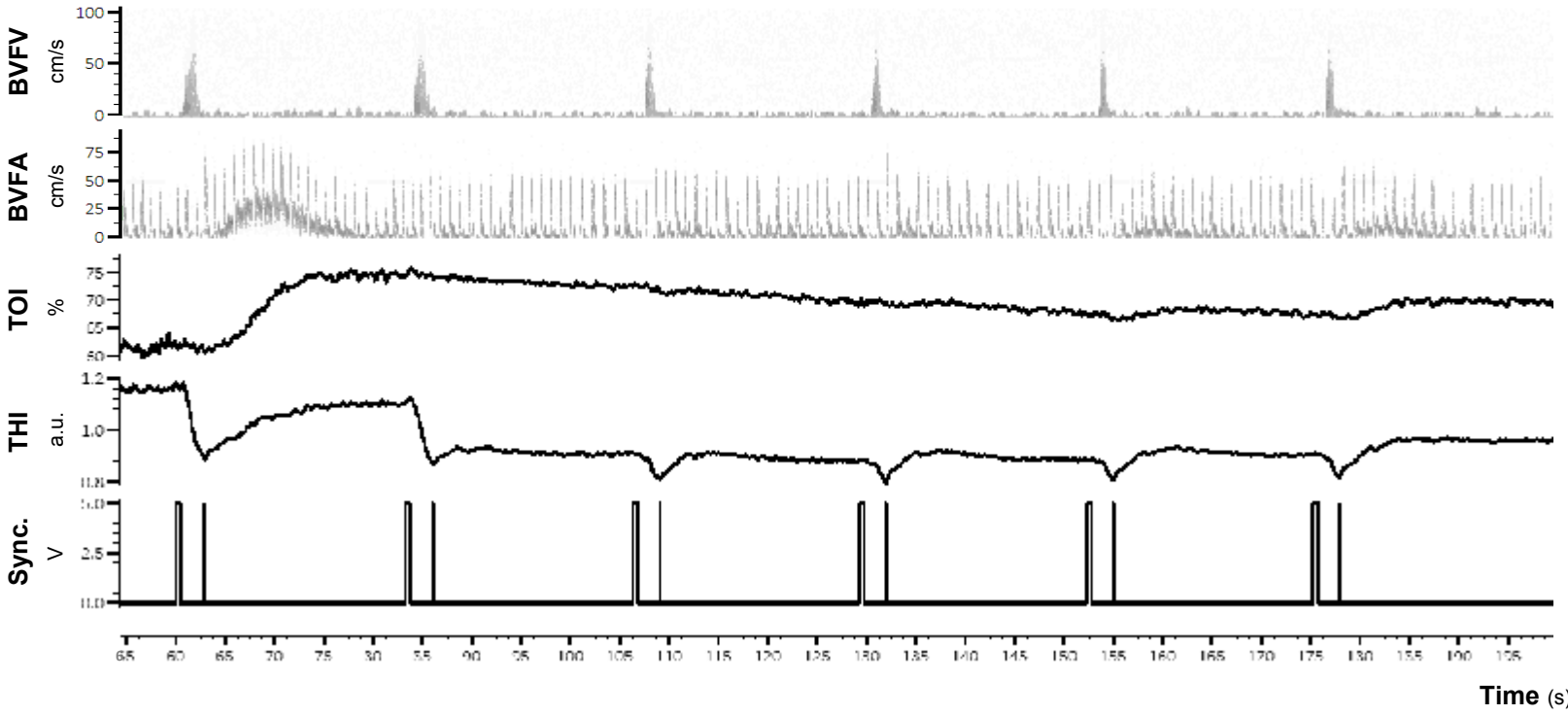

(Femoral artery and vein)
Doppler
IPC system
NIRS
(Gastrocnemius muscle)

B)

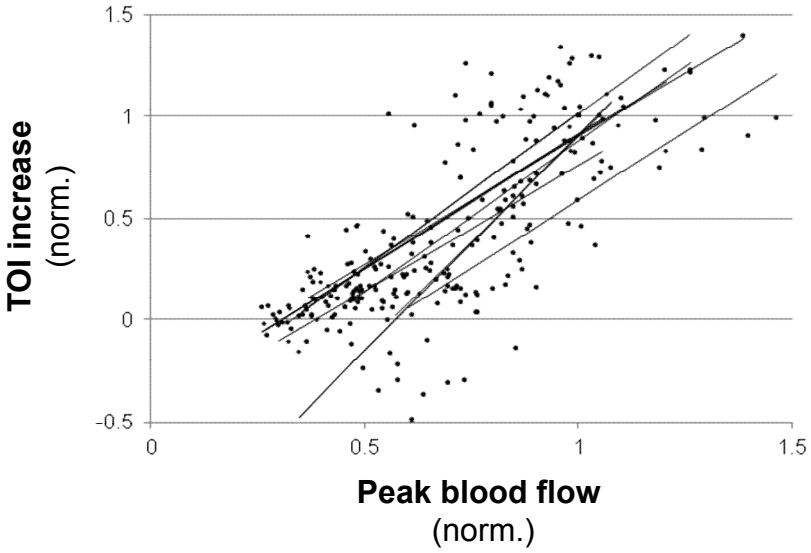

BVFV cm/s
BVFA cm/s
TOI %
THI a.u.
O,HB µM*cm
HHB µM*cm
Sync. V
Time (s)


Peak blood flow (norm.)
Displaced blood volume (norm.)
Pre-Compression THI (a.u.)
Pre-Compression TOI (%)
ISI (s)
160
80
60
40
20

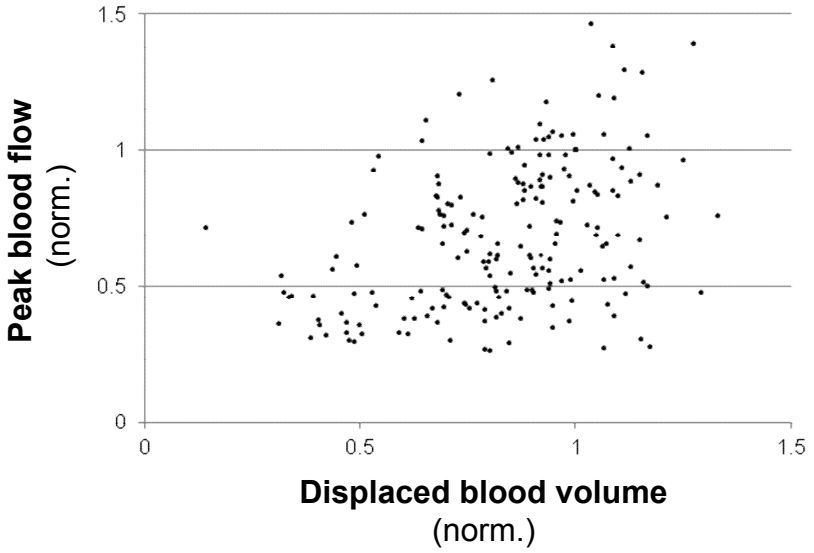

BVFV
cm/s
100
50
0
BVFA
cm/s
50
25
0
TOI
%
80
75
70
65
THI
a.u.
1.2
1.1
1.0
0.9
Sync.
V
5.0
2.5
0.0
220
230
240
250
260
270
280
290
300
310
320
330
340
350
360
370
380
390
400
410
420
430
440
Time (s)


BVFV
cm/s
100
50
0
BVFA
cm/s
75
50
25
0
TOI
%
75
70
65
60
THI
a.u.
1.2
1.0
Sync.
V
2.5
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
150
155
160
165
170
175
180
185
190
195
Time (s)

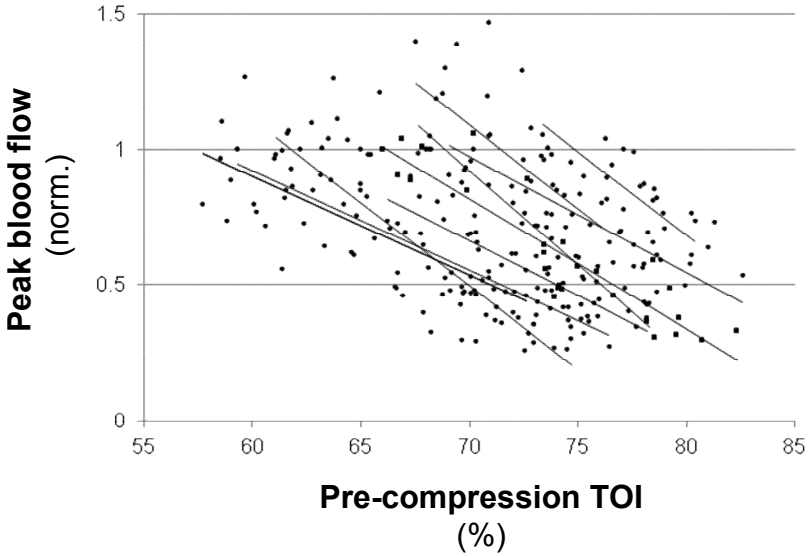
A)


TOI increase
(norm.)
1.5
1
0.5
0
-0.5
0
0.5
1
1.5
Peak blood flow
(norm.)

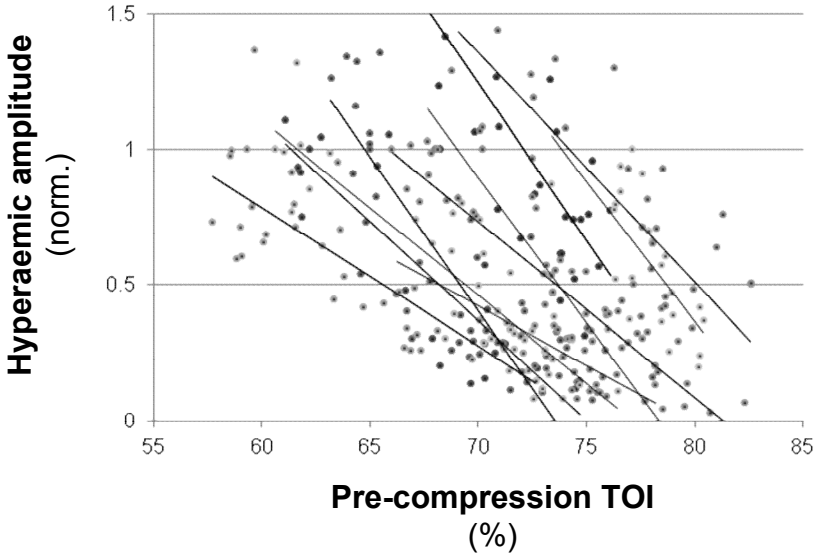
B)


Peak blood flow
(norm.)
1.5
1
0.5
0
0
0.5
1
1.5
Displaced blood volume
(norm.)

C)


Peak blood flow
(norm.)
1.5
1
0.5
0
55
60
65
70
75
80
85
Pre-compression TOI
(%)

D)


Hyperaemic amplitude
(norm.)
1.5
1
0.5
0
55
60
65
70
75
80
85
Pre-compression TOI
(%)