JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2017, 68, 3, 427-437
www.jpp.krakow.pl

A. MESSERE, M. TURTURICI, G. MILLO, S. ROATTA

REPETITIVE MUSCLE COMPRESSION REDUCES VASCULAR MECHANO-SENSITIVITY AND THE HYPEREMIC RESPONSE TO MUSCLE CONTRACTION

Department of Neuroscience, University of Torino, Torino, Italy

Animal studies have shown that the rapid hyperemic response to external muscle compression undergoes inactivation upon repetitive stimulation, but this phenomenon has never been observed in humans. The aim of the present study was to determine whether 1) the vascular mechano-sensitivity underlying muscle compression-induced hyperemia is inactivated in an inter-stimulus interval (ISI)-dependent fashion upon repetitive stimulation, as suggested by animal studies, and 2) whether such inactivation also attenuates contraction-induced hyperemia. Brachial artery blood flow was measured by echo Doppler sonography in 13 healthy adults in response to 1) single and repetitive cuff muscle compression (CMC) of the forearm (20 CMCs, 1 s ISI); 2) a sequence of CMC delivered at decreasing ISI from 120 to 2 s; and 3) electrically-stimulated contraction of the forearm muscles before and after repetitive CMC. The peak amplitude of hyperemia in response to CMC normalized to baseline decreased from 2.2 ± 0.6 to 1.4 ± 0.4 after repetitive CMC and, in general, was decreased at ISI < 240 s. The peak amplitude of contraction-induced hyperemia was attenuated after as compared to before repeated CMC (1.7 ± 0.4 and 2.6 ± 0.6, respectively). Mechano-sensitivity of the vascular network can be conditioned by previous mechanical stimulation, and such preconditioning may substantially decrease contraction-induced hyperemia.

Key words: *exercise hyperemia, mechano-sensitivity, muscle blood flow, rapid dilatation, muscle compression, inter-stimulus interval*

INTRODUCTION

Blood flow response to a single mechanical stimulus applied to a muscle or a group of muscles, e.g., by brief compression of a limb, can mimic the rapid hyperemia induced by a brief muscle contraction in both human and animal models (1-4). A similar hyperemic response can also be induced by briefly occluding the artery supplying the muscle, which reduces transmural pressure in downstream vessels, and by stretching the passive muscle (4). These observations support the idea that a common mechano-sensitive mechanism underlies such responses. Whether repetitive delivery of mechanical stimuli (e.g., external compression using a cuff applied to the forearm or leg) results in an increased or attenuated hyperemic response has been little investigated. Repetitive (n = 5) compression was observed to slightly reduce hyperemic response in the human forearm, although the effect did not reach statistical significance (3), while the same stimulation pattern enhanced the dilatory response of isolated arteries, indicating an opposite effect (5). When a longer sequence of compressive stimuli (n = 20) was applied in an animal model, the initial hyperemia was noted to rapidly fade away, in spite of continuing stimulation, and the extent of attenuation appeared to depend on the duration of the inter-stimulus interval (ISI) (6). Inactivation of the mechano-sensitive mechanism was suggested to underlie this phenomenon (6).

Surprisingly, this phenomenon has never been described in other animal (7) or human studies (8-12) investigating repetitive compressive stimuli, except for an early report by Tschakovsky *et al.* (2) in which attention was focused on the role of the muscle pump. Whether the muscle pump plays a role in the compression- and contraction-induced hyperemia is currently debated (13-15); however, its role could be contemplated in the attenuation of hyperemia during repetitive compression. Attenuated hyperemia may result from reduced filling of the vascular bed due to reduced muscle pump efficacy. Hence, the muscle pump constitutes a confounding factor when attempting to detect possible changes in active vascular response to compression (mechano-transduction).

Based on our previous findings in the animal model (6), we hypothesized that the same effects could also be observed in humans, i.e., that the hyperemia induced by cuff muscle compression (CMC) would be markedly reduced at the end of a sequence of 20 consecutive CMCs and that attenuation of the response would depend on the inter-stimulus interval. We then reasoned that repetitive CMC could be used as a tool to reduce mechano-sensitivity in the vascular network, a feature that could improve our understanding of the role of mechano-sensitive versus metabolic mechanisms in muscle blood flow regulation. We further hypothesized that, since the hyperemic response to muscle contraction has a mechano-sensitive basis (1, 16, 17), it would be attenuated by preconditioning the vascular network with repetitive CMC.

Exploring the mechanisms behind CMC hyperemia and its maintenance has broad implications for understanding the mechano-sensitive basis of hyperemia at the beginning of physical

exercise, as well as for the correct application of therapies and treatments based on repetitive limb compression, as was recently highlighted (18). The aim of the present study was to investigate by means of three experimental protocols: A) the residual hyperemia at the end of repetitive CMC (n = 20) as compared to the hyperemic response to single CMC; B) CMC delivered at increasing and then decreasing rates to determine the effect of the ISI on hyperemic response to CMC; C) the hyperemic response to an electrically stimulated muscle contraction (ESMC) compared before and after delivery of repetitive CMC. To exclude potentially confounding effects of the muscle pump, all measurements were taken on the forearm vertically positioned above heart level (2, 3).

MATERIALS AND METHODS

Subjects

The data from 2 of the 15 healthy subjects recruited for the study were discarded because of lack of hyperemic response to CMC. The final analysis was conducted on the remaining 13 subjects (11 men and 2 women; age 29.4 ± 8.2 years; weight 72.1 ± 8.5 kg; height 175.1 ± 8.2 cm) who completed the experimental protocols. All were non-smokers, non-obese (body-mass index (BMI, weight in kilograms divided by height in meters squared) < 25), normotensive (resting blood pressure < 140/90 mm Hg); none was taking medications. The experiments were performed in the morning at a room temperature of 22 – 24°C.

The study was approved by the local ethical committee (Prot. # 60195) of our institution, and all procedures complied with the principles of the Declaration of Helsinki. Subjects gave their written informed consent after having received an explanation of the purpose and procedures of the study.

Hemodynamic measurements

The experimental set-up is illustrated in *Fig. 1*. The subject lay supine with the experimental arm abducted at about 60 degrees and the forearm in vertical position with the elbow slightly raised above heart level. In this position, the blood pressure in forearm veins tends to drop below 0 mm Hg due to hydrostatic gradients, leading to venous emptying and collapse. This strategy has been previously used to minimize changes in the arteriovenous pressure gradient during CMC (i.e., the muscle pump) (2, 3). A humid glove made of thin cotton tissue and a fan directing air to the hand were used to cool the hand and reduce blood flow in that compartment (3). Changes in blood flow to forearm muscles were measured by monitoring blood velocity from the brachial artery with an echo Doppler device (MyLab 25, Esaote SpA, Genoa, Italy). The probe was positioned 5 cm proximally to the elbow inclined 45° with respect to the brachial artery.

Forearm compression

A custom-made compression system provided controlled and repeatable compressive stimuli to the forearm. The system

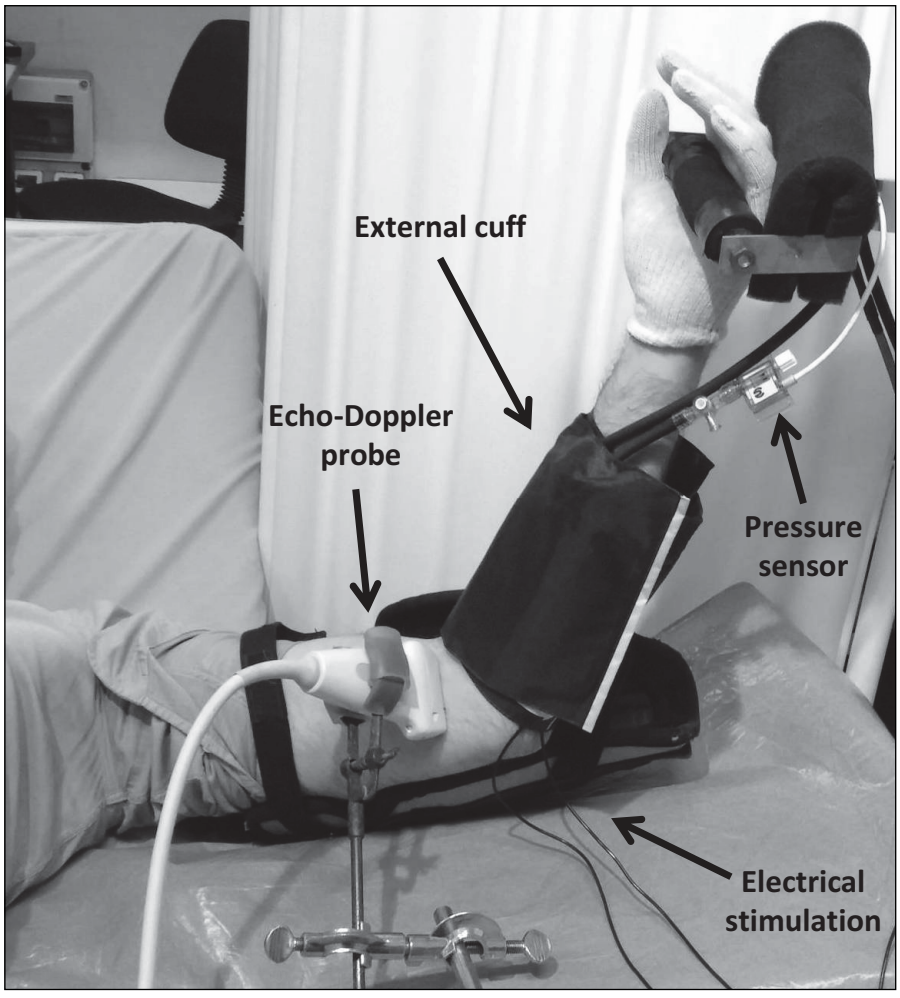

Fig. 1. Experimental set-up. Note the location of the pressure sensor at the outlet of the cuff.

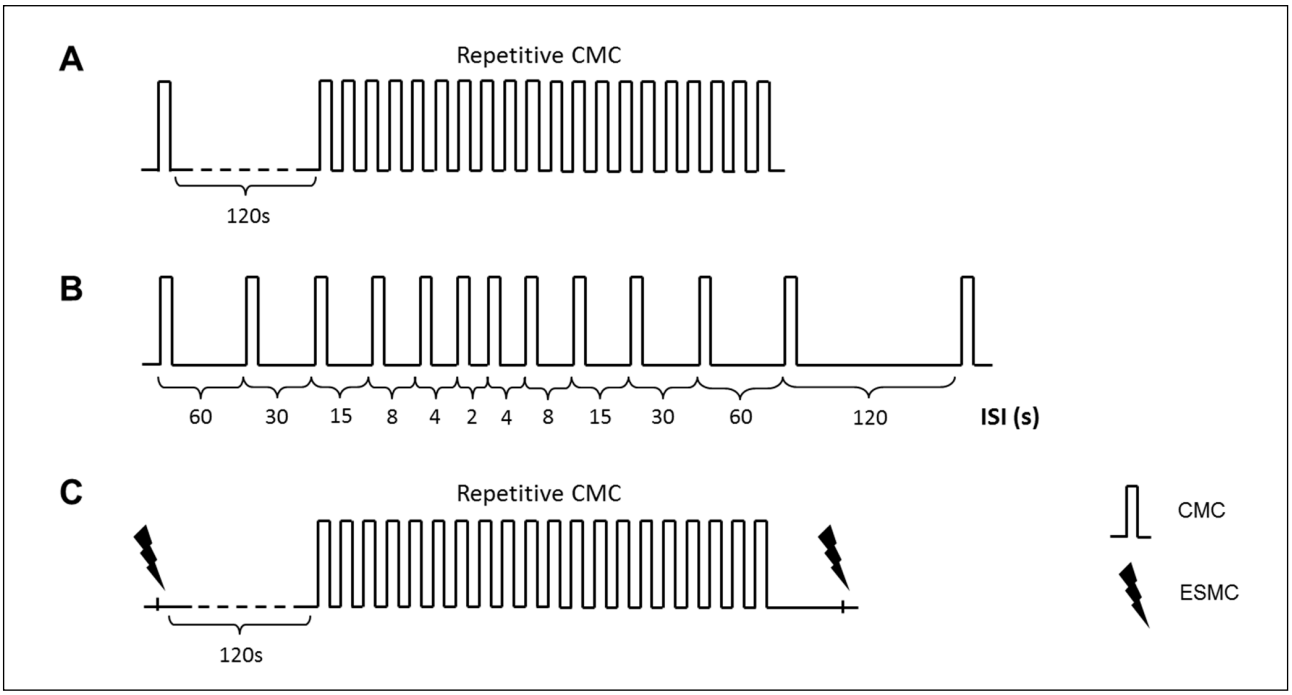

Fig. 2. Experimental protocols. (*A*) Single and repetitive cuff forearm compression (CMC, 150 – 200 mm Hg, 1 s ON – 1 s OFF, duration 40 s). (*B*) CMC delivered at inter-stimulus intervals (ISI) progressively decreasing and increasing from 120 to 2 to 120 s. (*C*) Single electrically stimulated muscle contraction (ESMC) elicited before and a few seconds after repetitive CMC (150 – 200 mm Hg, 1 s ON – 1 s OFF, duration 40 s).

consisted of a cuff wrapped around the forearm which could be rapidly inflated by an air compressor and deflated by a vacuum pump. Inflation and deflation timing was accurately controlled by PC-driven electro-valves that generated short compressive stimuli with adjustable rise and falling times and plateau duration. The digital signals driving the electro-valves were designed with Spike2 software (Cambridge Electronic Design, Cambridge, UK) and generated by a data acquisition board (CED Micro 1041, Cambridge Electronic Design): inflation time 0.15 s; plateau duration 1 s; deflation time 0.5 s. These time parameters were set based on the protocols previously tested in the rabbit model (6), in which brief compression is repetitively delivered at a high rate (see below).

A pressure sensor at the cuff outlet and connected to a pressure monitor (pressure monitor BP-1,WPI, Inc., Sarasota, FL, USA) monitored the time course and reproducibility of the actual compression stimulus delivered. Pressure level at plateau (range 150 – 200 mm Hg) was obtained by adjusting the air pressure delivered by the compressor. This pressure level was chosen since compression at pressures between 100 and 200 mm Hg were shown to elicit stereotyped hyperemic responses at nearly maximal amplitude (3, 19).

Muscle contraction

In order to obtain reproducible levels of contraction intensity and duration, electrically evoked rather than voluntary contractions were induced. Electrical stimulation (frequency 20 Hz, duration 1 s; pulse duration 0.5 ms) was delivered by an electrical stimulator (Digitimer, model DS7A, Digitimer, Welwyn Garden City, Herts, UK) *via* two electrodes (4 cm × 4 cm) positioned on the ventral and dorsal aspects of the right forearm. Based on the results of preliminary tests, the current was adjusted to produce the strongest contraction with minimal discomfort for the subject. The electrodes were placed underneath the cuff without discomfort during the compressive action.

Experimental protocol

The experiment started after 20 minutes of rest with the subject lying in supine position. The three protocols were performed in random order: A) single CMC, followed by 2 minutes rest and a series of 20 CMCs delivered with an inter-stimulus interval (ISI) of 2 s (repetition frequency 0.5 Hz; *Fig. 2A*); B) a series of CMCs delivered at different ISI (> 120, 60, 30, 15, 8, 4, 2, 4, 8, 15, 30, 60, 120 s; *Fig. 2B*); C) ESMC delivered 2 minutes before and a few (5 – 10) seconds after a series of 20 CMCs (ISI 2 s; *Fig. 2C*). Resting intervals of at least 120 s were allowed between each experimental protocol since preliminary experiments showed that this interval is adequate for full recovery of vascular response.

Additional trials were performed in a subset of subjects (n = 6) to determine whether the CMC series *per se* could alter the diameter of the insonated brachial artery and whether the response to a single CMC was as affected by the CMC series as the response to the ESMC. To this aim:

A) the diameter of the brachial artery was measured by 15-s M-mode video recording before and after a 20-CMC series.

B) a single CMC instead of ESMC was delivered before and after the 20-CMC series according to a modified protocol (protocol C).

Data acquisition and processing

The data acquisition board was also used to acquire Doppler audio signals (sampling frequency 12000 Hz) and cuff pressure (100 Hz). A specific algorithm was implemented in

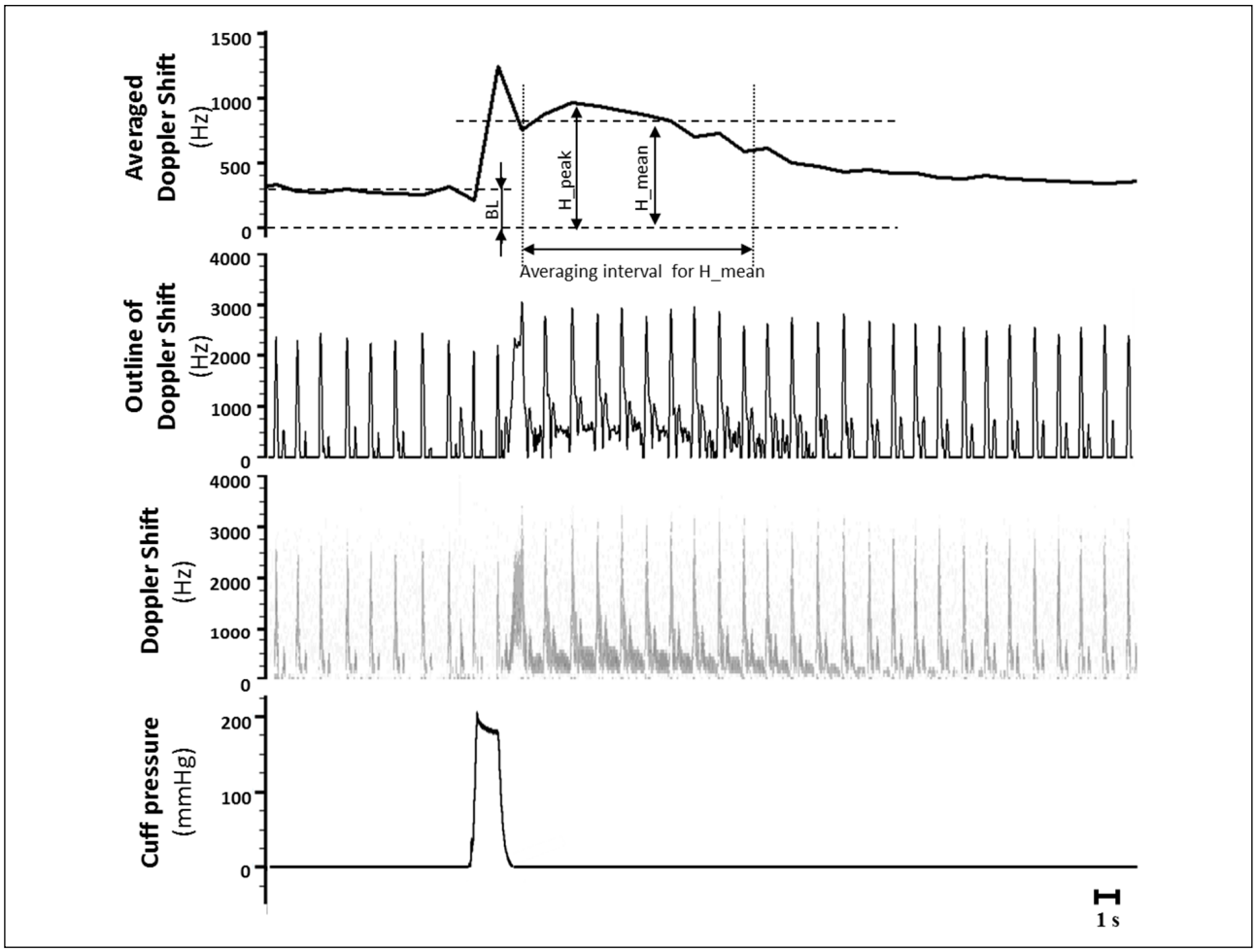

Fig. 3. Representative example of the response to cuff forearm compression (CMC). From bottom to top, the fast pressure transient at the forearm cuff (bottom), blood velocity (Doppler shift) measured at the brachial artery, outline of the Doppler shift averaged over single cardiac cycles. Indication of the measurements is superimposed on the upper tracing: baseline (Bl), peak (H_peak), and mean (H_mean) of the hyperemic response. The immediate velocity peak occurring at release of compression was not included in the analysis.

the Spike2 (version 6.10, Cambridge Electronic Design) script language to calculate blood velocity from the Doppler audio signal (20, 21). Briefly, the outline of the Doppler shift (related to maximum blood velocity) was detected from the power spectrum of the Doppler signal computed over 15-ms epochs and then time-averaged over each cardiac cycle (see example in *Fig. 3*). In order to assess changes in the amplitude and duration of the hyperemic response to CMC and to ESMC, the peak amplitude (H_peak) and the mean value (H_mean) attained over a fixed time interval (8 s for CMC and 10 s for ESMC) were measured. The first cardiac cycle after cuff deflation was excluded from the analysis (*Fig. 3*). The H_mean was computed in addition to the H_peak in order to account for possible changes in duration of the hyperemic response. Since preliminary experiments indicated a slightly longer-lasting hyperemic response to ESMC than to CMC, a longer interval for H_mean was adopted for ESMC. The H_mean was not computed for ISI < 15 s in protocol B. These parameters were evaluated in relative terms, with respect to the basal level, averaged over a 20-s interval preceding the first stimulus delivered. The diameter of the brachial artery was calculated from the average of three different readings obtained from the 15-s M-mode video recording.

Statistical analysis

Student's t-test was used to compare the mean and peak amplitude of the hyperemic response to single CMC with the response measured at the end of the 20-CMC series (protocol A). One-way ANOVA for repeated measurements and the Dunnett post-hoc test were applied to the changes in response to CMC delivered at different ISI as compared with the response to the first CMC in the series (protocol B). Student's t-test was used to compare the responses to ESMC before and after the 20-CMC series (protocol C). All values are reported as mean ± standard deviation (expressing inter-subject variability) in the text and mean ± standard error in the figures.

RESULTS

Protocol A - response to single and repetitive muscle compression

Examples of the classical response to single CMC are shown in *Figs. 3* and *4A*. Blood velocity immediately increased after the end of stimulus and peaked in 3 – 4 cardiac cycles (4

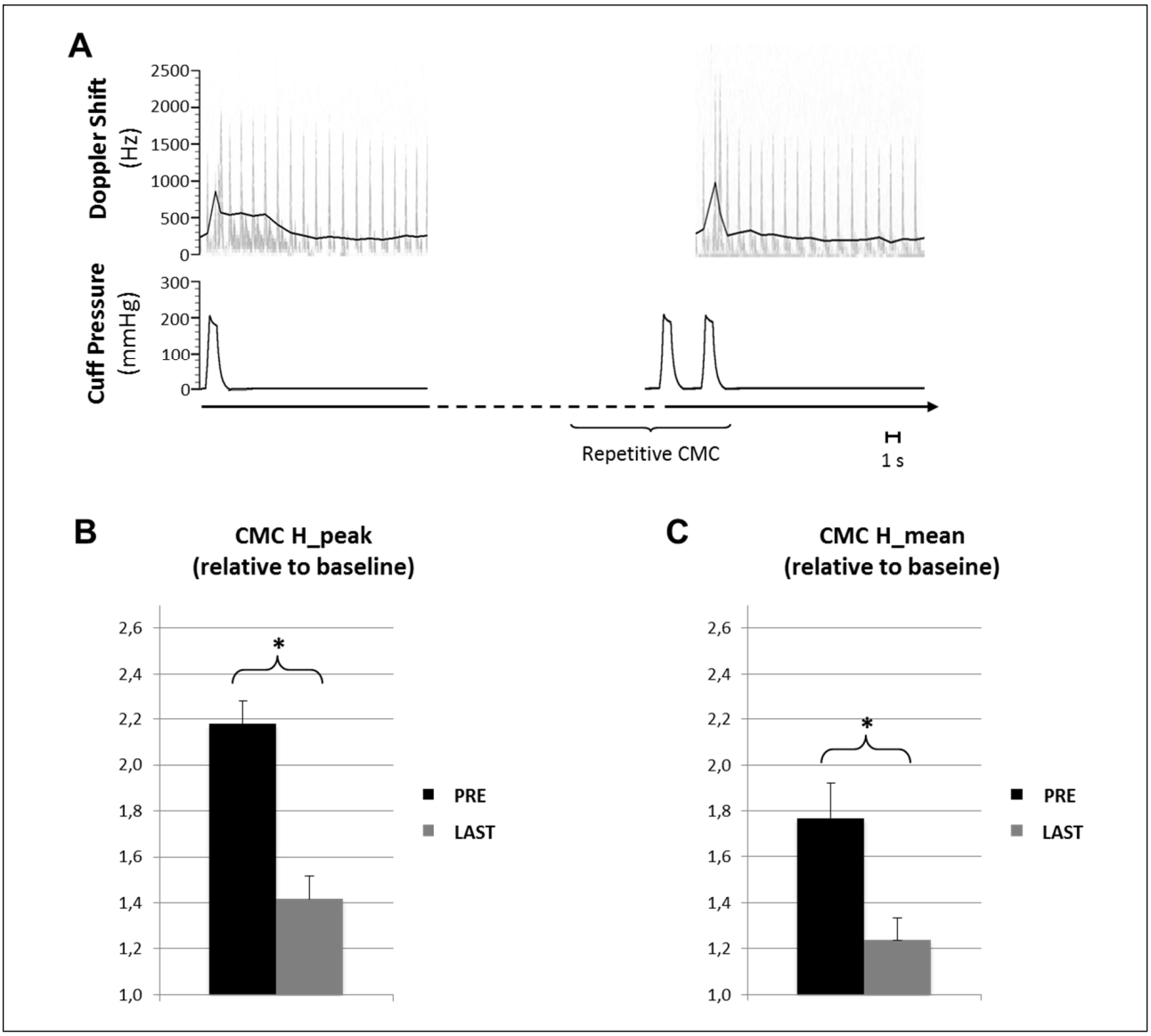

Fig 4. Response to single and repetitive cuff muscle compression (CMC) (Protocol A). Representative examples of the hyperemic response to CMC, before (single, left) and at the end (last, right) of repetitive CMC (*A*); the averaged outline of the Doppler shift is superimposed (black line). Average peak (CMC H_peak, *B*) and mean (CMC H_mean, *C*) amplitude of the hyperemic response to the single and the last CMC in the series, normalized to pre-stimulus level (baseline). *P < 0.01. Error bars represent standard error.

– 5 s) before returning to basal level within about 20 s. There was an immediate increase in blood velocity during compression due to vessel collapse (i.e., the first peak observed) and a subsequent, more slowly developing hyperemia (*Fig. 3*). On average (n = 13), blood velocity reached a peak (CMC H_peak) of 2.2 ± 0.6 and a mean of 1.8 ± 0.5 (CMC H_mean) relative to baseline (5.0 ± 1.4 cm/s) (black bars in *Fig. 4B* and *4C*).

In comparison, the hyperemic response elicited by the last CMC in the sequence of 20 compressions was markedly reduced in both amplitude and duration (*Fig. 4A*). On average, the relative blood velocity decreased to 1.4 ± 0.4 (CMC H_peak; P < 0.01) and 1.2 ± 0.3 (CMC H_mean; P < 0.01) (grey bars in *Fig. 4B* and *4C*). Given that the relative blood velocity equal to 1 corresponds to baseline (no hyperemia), the peak and mean of the hyperemic response were reduced by 60% and 83%, respectively.

Protocol B - compressive stimulation at increasing and decreasing rates

With this stimulation protocol we wanted to determine whether the hyperemic response to CMC is affected by progressively decreasing the ISI from 120 to 2 s and then increasing it back to 120 s (*Fig. 2B*). On average, the amplitude of the hyperemic response was reduced at low ISI, the largest effect occurring when switching from 120 to 60 s. At an ISI of 30 s, attenuation of the hyperemic response was about 40% and 50% for the CMC H_peak and CMC H_mean values, respectively. The measurements presented in *Fig. 5* are normalized to the initial baseline value (4.9 ± 1.0 cm/s) at the beginning of the protocol and were not affected by changes in the baseline between stimuli. However, the baseline remained within 80% and 120% of its initial value for all intervals between 15 s < ISI < 120 s. The response to CMC tended to recover when

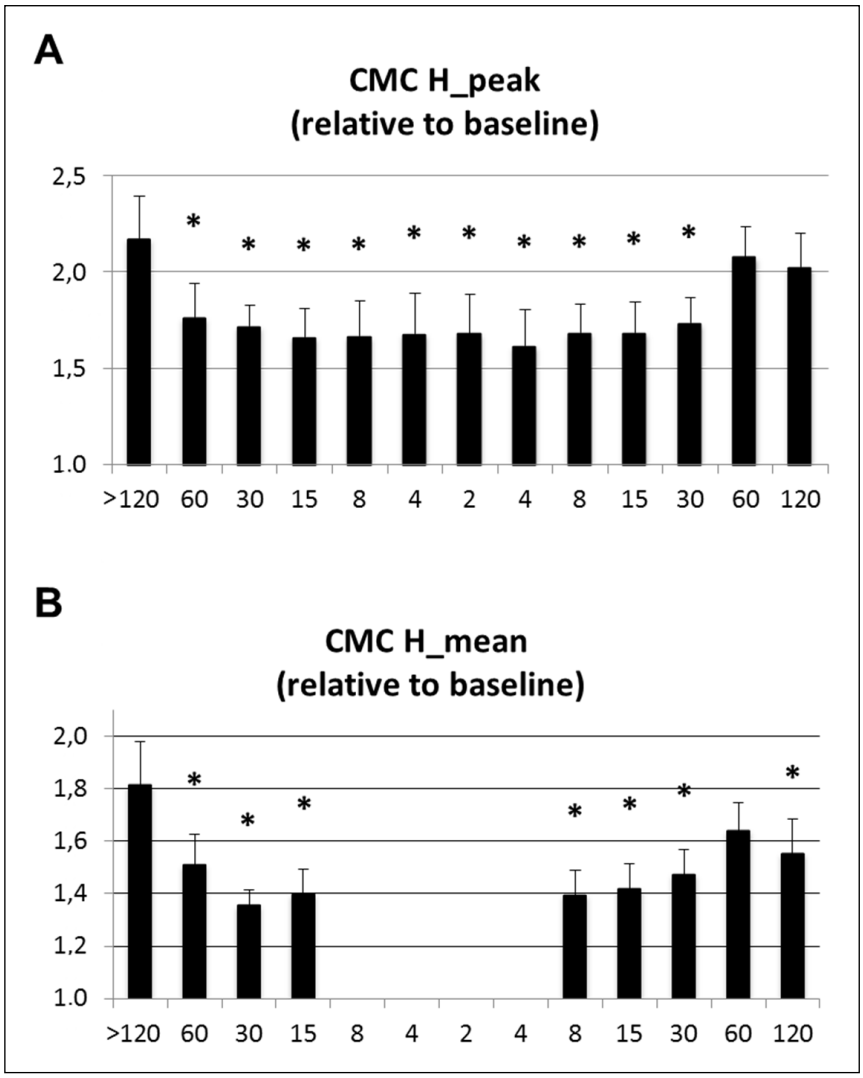

Fig. 5. Response to cuff muscle compression (CMC) at different inter-stimulus intervals (ISI) (Protocol B), assessed in terms of peak (CMC H_peak, *A*) and mean amplitude (CMC H_mean, *B*), normalized to baseline. Since the H_mean could not be measured over time intervals < 8 s, the central bars are missing in *B*). *significantly different from the first bar (ISI ≥ 120 s), P < 0.01).

ISI was increased back to 120 s, at least in terms of peak amplitude.

Protocol C - single stimulation before/after repetitive compression

The effects of repetitive CMC were tested on the hyperemic response to ESMC. An example of the hyperemia induced by an ESMC is presented in *Fig. 6A*. On average, the peak and mean amplitude of blood velocity was 2.6 ± 0.6 and 2.1 ± 0.6, respectively, relative to baseline (4.7 ± 1.1 cm/s). The response was considerably reduced when ESMC was performed a few seconds after the delivery of a series of 20 CMCs. On average, the peak and mean blood velocity was reduced to 1.7 ± 0.4 (P < 0.01) and 1.3 ± 0.4 (P < 0.01), respectively (*Fig. 6B* and *6C*), with a corresponding reduction of 53% and 72% in hyperemic response.

Additional trials

A) No significant difference in brachial artery diameter before (0.46 ± 0.14 cm) and after (0.45 ± 1.3 cm) delivery of a 20-MC sequence was observed in the subset of subjects (n = 6).

B) In this modified version of protocol C, single CMC stimuli instead of ESMC were delivered to test the effect of repetitive CMC on the response to a single CMC. Peak and mean blood velocity decreased from 1.9 ± 0.8 and 1. 5 ± 0 .7 to 1.4 ± 0.5 (P < 0.01) and 1.1 ± 0.5 (P < 0.01), respectively, corresponding to a reduction of 59% and 81%, respectively, in peak and mean hyperemic response (n = 6).

DISCUSSION

The present study yielded several new findings: 1) for the first time it was observed in human subjects that the rapid hyperemic response to mechanical stimulation is reduced following stimulation in an ISI-dependent manner, as previously observed in an animal model; 2) repetitive compression can be used as a tool to transiently reduce the mechano-sensitivity of the vascular network; 3) preventive exposure to repetitive compression markedly attenuates the hyperemic response to a short-lasting contraction.

Studies investigating vascular response to repeated mechanical stimulation have reported conflicting results. Kirby *et al*. (3) observed that the response to five consecutive cuff compressions of the forearm (200 mm Hg, 2-s ON, 1-s OFF) was non-significantly attenuated by about 26% as compared with the response to a single compression. Conversely, Clifford *et al*. (5) delivered five consecutive compressive stimuli on

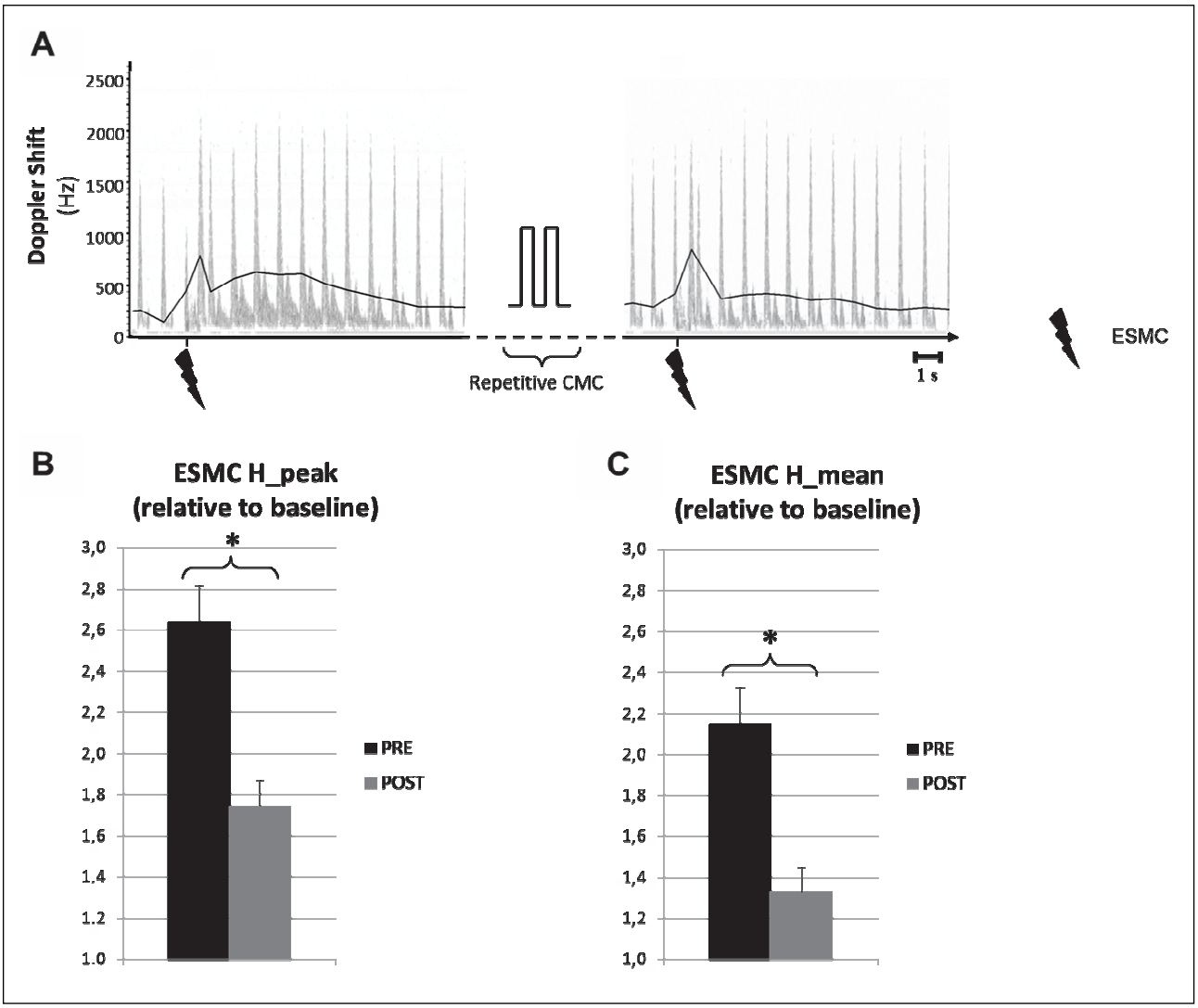

Fig. 6. Response to electrically stimulated muscle contraction (ESMC) before and after repetitive cuff muscle compression (Protocol C). (*A*) representative original recordings, the averaged outline of the Doppler shift is superimposed (black line); average peak (ESMC H_peak, (*B*) and mean (ESMC H_mean (*C*)) amplitude of the hyperemia. Responses were individually normalized to resting blood velocity level. *P < 0.01. Error bars indicate standard error.

isolated feed arteries from the rat soleus muscle (600 mm Hg delivered to the chamber containing the isolated artery, 1-s ON, 1-s OFF) and observed a significant increase in dilatory response as compared with single compression. In both studies, the effect was assessed at the end of the stimulation sequence, as we did in Protocol A.

More recently, a model was developed in which blood flow in the masseteric artery (22) was monitored in response to masseter compression exerted by an external head (diameter 1.3 cm) directed to the cheek in the anesthetized rabbit (19). In this model progression of the hyperemic response was monitored not only at the end but also during repetitive mechanical stimulation (6). This was possible owing to the rapid heart rate of the animal (typically 240 bpm), developing 3 – 4 full cardiac cycles between compressive stimuli, and it allowed us to detect attenuation of hyperemia, beginning at 4 – 6 s after stimulation onset, progressing in spite of continuing stimulation, and return of blood flow to nearly basal level at the end of a compressive series (20 1-s compressions delivered at 0.5 Hz) (6). In addition, it was shown that the amplitude of the hyperemic response to single mechanical stimulation depended on the ISI and was decreased up to 70% at an ISI of 2 s (6). These findings from the animal model are shared by those of the present study in humans. Although it was not possible to monitor perfusion during repetitive CMC, the hyperemia at the end of the same compressive series was markedly reduced as compared with the single CMC (*Fig. 4A* and *4B*). CMC-induced hyperemia was also observed to decrease at low ISI and to recover when the ISI was returned to 60 – 120 s, although the extent of reduction in the amplitude of response was less in humans (40%) as compared to the rabbit. While this quantitative difference may be attributed to species-related differences and methodological aspects, the qualitative similarity between the results obtained in the animal and human models strongly supports the validity of the animal model for the investigation of vascular reactivity (19, 22). Importantly, blood flow exclusively supplying muscle tissue was recorded in the rabbit (6), whereas mixed cutaneous-muscular blood flow, also including contributions from non-stimulated areas (e.g., the hand) was recorded in the present and previous human studies. This aspect is relevant, considering the

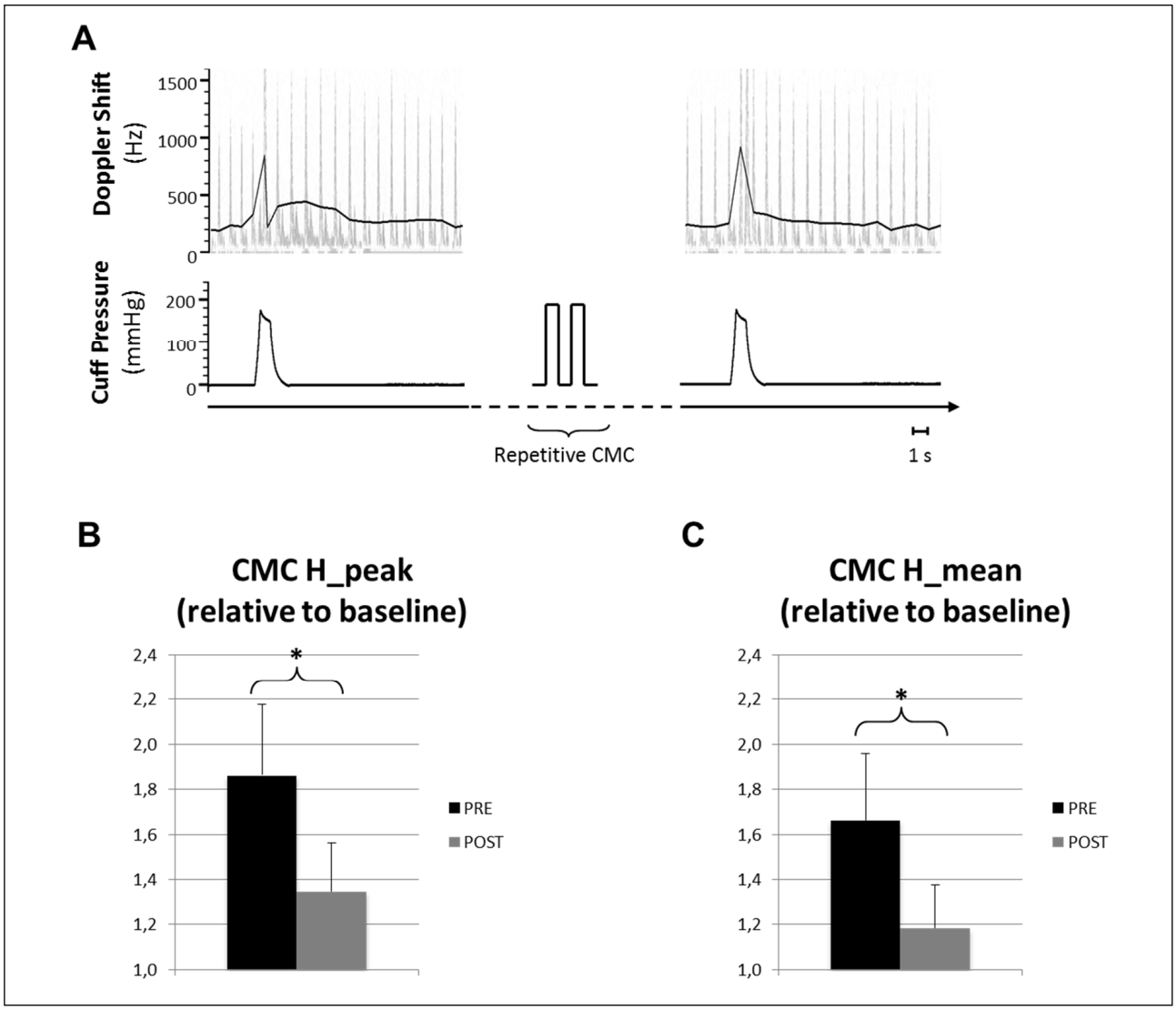

Fig. 7. Response to single cuff muscle compression (CMC) before and after repetitive cuff muscle compression (Additional trial b). (*A*) representative original recordings, the averaged outline of the Doppler shift is superimposed (black line); average peak (CMC H_peak, (*B*) and mean (CMC H_mean (*C*)) amplitude of the hyperemia. Responses were individually normalized to resting blood velocity level. *P < 0.01. Error bars indicate standard error.

fact that the cutaneous vascular bed is less responsive to mechanical stimulation than the muscular vascular bed (4).

No involvement of the muscle pump

Hyperemic response to single and repetitive compression has been shown to be larger when the arm (or, more generally, the limb) is maintained below the heart level, presumably due to an increase in the arteriovenous pressure gradient during venous emptying (muscle pump) (2, 10, 23). One could speculate that at low ISI (Protocols A and B) there is not enough time for the venous compartment to re-fill, thus attenuating muscle pump efficacy and hyperemic response to forearm compression. However, the muscle pump mechanism is not activated when the forearm is maintained vertically above the heart level, as seen in the present study, because the forearm veins are already completely collapsed (2, 3, 23). On this basis, the decreased hyperemia we observed in response to the last compressive stimulus in the series, as compared with the single (Protocol A) or in response to compressive stimuli at low ISI (< 60 s), as compared to high ISI (> 60 s) (Protocol B), cannot be attributed to a reduced efficacy of the muscle pump. The additional trials support the same conclusion: allowing for 5 – 10 s of vascular refilling at the end of repetitive compression does not change the attenuation in the hyperemic response to CMC, as compared to protocol A.

Human studies investigating long-lasting repetitive compression treatments

Recent studies have reported an increase in average blood flow during ongoing repetitive compression at 4 – 5 minutes from the beginning, with respect to resting blood flow. Sheldon *et al*. (8) reported a 200% increase in popliteal blood flow during pneumatic compression of the foot and calf, both at 'low' (ISI 20 s) and 'high' (ISI 5 s) stimulation frequency in subjects in the upright sitting position, while Crecelius *et al*. (24) reported an increase in forearm blood flow of about 30%

during repetitive compressions (ISI 3 s) in subjects in supine position. Their observations are in line with our results obtained in protocol B (*Fig. 5B*), i.e., the average hyperemic response was initially 180% then quickly decreased and stabilized at around 140% of baseline. The differences in the absolute values are likely related to differences in experimental conditions (leg/forearm below/above heart level, stimulation pattern, averaging interval).

Moreover, in light of the present and previous results from the animal model (6), we speculate that the authors might have missed a more prominent hyperemia developing at the very beginning of the compressive sequence and progressively 'adapting' to a lower level. This response pattern has occasionally been reported in earlier studies: Tschakovsky *et al*. (2) observed a prominent initial increase (+60%), later stabilizing at a lower level (+35%), during repetitive forearm compression (ISI 2 s) below heart level and a similar pattern of increase with the arm raised above the heart level (albeit not reaching statistical significance). Using a different stimulation pattern (sequence of compressions at 60 mm Hg, 10 s ON, 50 s OFF, i.e., ISI 60 s) Morris *et al*. (25) found that the hyperemic peak in response to the first stimulus in the sequence was about double that of the subsequent stimuli. Since it has been observed that CMC-induced hyperemia can be obtained at different pressure levels (50 – 300 mm Hg) (3, 19, 24) and compression durations (1 – 6 s) (3, 19), we believe that Morris *et al*. (25) reported an early observation of the attenuation effect described in the present study (see Protocol B, the ratio of the two leftmost columns of *Fig. 5A* is about 50%).

Attenuation of contraction-induced hyperemia

The present study shows for the first time that inactivation of mechano-sensitivity also attenuates the rapid hyperemia evoked by muscle contraction (Protocol C, *Fig. 6*). This is in line with previous reports and suggests that rapid dilatation at the onset of exercise is at least partly mediated by mechano-sensitive mechanisms (3, 4, 23, 26), although their actual contribution is still a matter of debate. Credeur *et al*. considered the contribution of mechanical factors almost negligible, as they found the peak response to a 1-s, 300 mm Hg compression (leg above heart level) to be only about 5% of the response to a 1-s contraction at 60% of maximal voluntary contraction (MVC) (23). On the other hand, Kirby *et al*. found that the peak response to 200 – 300 mm Hg compression (forearm above the heart level) was matched by a 1-s, 20% MVC (3). In the anesthetized rabbit, the hyperemic response to brief (3 – 400 ms) spontaneous contractions of the masseter muscle was mimicked by the response to several mechanical stimuli delivered to the passive muscle, such as compressive stimuli (< 80 mm Hg), brief occlusion of the supplying artery, and passive muscle stretching (jaw opening) (4). The rapid hyperemia induced by these mechanical stimuli was shown to decrease in spite of ongoing stimulation, in the case of brief muscle compression and brief occlusion of the supplying artery (4), as well as in the case of passive movement (27, 28). Obviously, this pattern of transient hyperemia is not observed with repeated muscle contraction, due to the superimposed functional hyperemia (29). However, the fact that the preconditioning operated by repetitive muscle compression is able to reduce by 70% the hyperemic response to a brief muscle contraction, just as it reduces the response to CMC, further supports the idea that the latter is largely dependent on mechano-sensitive mechanisms.

In the present study, contraction was elicited by electrical stimulation, some 5 – 10 s after the end of repetitive CMC. Stimulated contractions, which were adopted in order to obtain strictly reproducible responses, have often been used for investigating functional hyperemia (30, 31) and, in this respect, are considered to be equivalent to voluntary contractions (3).

Possible mechanisms underlying the attenuation of mechano-sensitive hyperemia

It was previously hypothesized (6) that attenuation of MC-induced hyperemia could be mediated by a rapid (< 1 s) and transient (few minutes) inactivation or desensitization of the relevant mechano-sensitive structures, e.g., TRAAK (TWIK-related arachidonic acid activated K^+ channel) or TRP (transient receptor potential) channels possibly located on the endothelium or vascular smooth muscle (32, 33). However, the mechano-sensitive structures responsible for initiating a rapid vasodilatory response have not yet been identified. The mechano-sensitive channel blocker Gd^{3+} only partially attenuated the hyperemic response to muscle compression (34), and several other pathways have been hypothesized to underlie the rapid dilatory response to muscle compression or to a short contraction, including erythrocytes, ATP, nitric oxide, potassium, and prostaglandins, but their precise role and interplay remain to be elucidated (17, 35).

An alternative hypothesis is that inactivation of vascular mechano sensitivity could result from local autoregulation that matches perfusion to metabolism. In this regard, CMC hyperemia leads to an alteration of the homeostatic levels of different variables, including O_2 and CO_2 partial pressures, and pH, that potentially elicit counter-regulatory reactions. Although the extent and timing of the constrictor response to hyperperfusion is not precisely known (6, 36), this hypothesis fits with the bang-bang model of autoregulation according to which increased tissue oxygen concentration triggers a constrictor response (37) mediated by the inhibitory action of superoxide on tonically released NO (38). Supporting this hypothesis is the observation that increased dilatation in response to five consecutive compressions was observed on isolated arteries *in vitro* (in the absence of tissue metabolic signals) (5) but not *in vivo* (3). On the other hand, no reduction in blood flow as a result of counter-regulation has ever been reported to follow compression-induced hyperemia in either the present or previous studies (3, 4). Further studies are needed to clarify the mechanism.

Functional implications

Irrespective of whether inactivation of vascular mechano-sensitivity occurs due to intrinsic properties of mechano-sensitive structures or to activation of feedback autoregulatory mechanisms, the transient nature of mechanically-induced hyperemia fits nicely with the functional meaning currently attributed to mechanically-stimulated dilatation, i.e., to provide a rapid initial feed-forward increase in muscle blood flow at the onset of exercise. Hyperemia is then be maintained only by an increase in the metabolic rate if exercise continues (3, 6, 26). Repeated mechanical stimuli unrelated to muscle activity (e.g., passive limb movement or external muscle compression) only produce a transient hyperemia, thus limiting passive muscle hyperperfusion and saving systemic resources.

Although the underlying mechanisms remain to be elucidated, this phenomenon has implications for the administration of massage and physiotherapy treatments to improve local circulation, as well as in intermittent pneumatic compression (IPC) of limbs, often used for the treatment of peripheral arterial disease. In a recent review, Sheldon *et al*. (18) emphasized the idea that, besides the increase in the arteriovenous gradient generated by the compression-induced emptying of venous compartments, mechano-sensitive dilatation plays a relevant role in the hyperemia

that develops during IPC treatments. On this basis, they also suggested that IPC protocols, which traditionally implement rather low stimulation frequencies (2 – 4 cycles per minute) to take advantage of the arteriovenous difference, should be revised (18). In this respect, the rapid reduction in mechano-sensitive dilatation upon repetitive muscle compression as documented in the present study is a further element to be considered when programming intermittent compressive treatment, if a prominent increase in perfusion is the aim of the treatment.

Limitations of the study

The lack of control of dietary intake of the subjects may have contributed to the individual variability of hyperemic responses, and possibly to the low responsiveness of the two subjects excluded from the study. Further studies will be necessary to investigate the dependence of vascular mechano-transduction on dietary intake. Furthermore, changes in brachial artery diameter were not systematically monitored. In a subset of subjects, however, changes were observed to be unaffected by repetitive forearm compression, in agreement with other studies that reported no changes in brachial diameter in similar conditions of transient and modest hyperemia (3, 39). Moreover, even a large (about 3 times the baseline) and sustained increase in blood velocity, as produced by intermittent handgrip exercise, does not produce significant increases in brachial artery diameter before 60 s from the beginning of exercise (40). On this basis, the changes in blood velocity (Doppler shift) observed can be interpreted in terms of changes in blood flow.

Conclusion

We observed for the first time in humans that 1) mechano-sensitive dilatation, underlying the hyperemic response to a compressive stimulus, is inactivated by previous repeated mechanical stimulation and that 2) the extent of inactivation depends on the inter-stimulus interval, a resting time on the order of about 2 minutes being necessary for complete functional recovery. In addition, our results show that 3) such inactivation also effectively attenuates the hyperemic response to brief muscle contraction, suggesting a major role for mechano-sensitive mechanisms in the hyperemia at the beginning of exercise.

Abbreviations: CMC, cuff muscle compression; ESMC, electrically stimulated muscle contraction; ISI, inter-stimulus interval; MVC, maximal voluntary contraction; IPC, intermittent pneumatic compression

Acknowledgments: This study was supported by grants from the University of Torino and the Italian Ministry of Heath (RF-2009-1551299).

Conflict of interests: None declared.

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Received: January 13, 2017
Accepted: May 30, 2017

Author's address: Prof. Silvestro Roatta, Integrative Physiology Laboratory, Department of Neuroscience, University of Torino, c.so Raffaello 30, 10125 Torino, Italy.
E-mail: silvestro.roatta@unito.it