Timed Synchronization of Muscle Contraction to Heart Beat Enhances Muscle Hyperemia

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Running title: Synchronized muscle contractions to heartbeat

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New & Noteworthy: When muscle contraction is synchronized with the pulsed delivery of blood flow to active muscle, muscle functional hyperemia can be either maximized or minimized. This suggests a possibility to couple different strategies to enhance the acute and chronic effects of exercise on the cardiovascular system.

Abstract (words: 250)

Blood flow (BF) to exercising muscles is susceptible to variations of intensity, and duration of skeletal muscle contractions, cardiac cycle, blood velocity, and vessel dilation. During cyclic muscle activity, these elements may change proportionally with or without direct optimal temporal alignment, likely influencing BF to active muscle. Ideally, the pulsed delivery of blood to active muscle timed with the inactive phase of muscle duty-cycle would enhance the peak and average BF. To investigate the phenomenon of muscle contraction and pulse synchronicity, electrically-evoked muscle contractions (trains of 20Hz, 200ms duration) were synchronized with each systolic phase of the blood velocity spectrum (aBVS). Specifically, unilateral quadriceps contractions matched in-phase (IP) with the aBVS were compared to contractions matched out of phase (OP) with the aBVS in 10 healthy participants (26±3 years). During each trial, femoral BF of the contracting limb and central hemodynamics were recorded for 5 minutes with an ultrasound doppler, a plethysmograph, and a cardioimpedence device. At steady-state (5^{th} min) IP BF (454±30 mL/min), MAP (108±2 mmHg) and vascular conductance (4.3±0.2 mL/min/mmHg) were significantly lower (all, $P<0.001$) in comparison to OP BF (784±25 mL/min), MAP (113±3 mmHg) and vascular conductance (6.7±0.2 mL/min/mmHg). On the contrary, no significant difference (all, $P>0.05$) was observed between IP and OP central hemodynamics (HR:79±10 vs. 76±11 bpm, CO:8.0±1.6 vs. 7.3±1.6 L/min) and ventilatory patterns (V_E:14±2 vs. 14±1 L/min, VO_2:421±70 vs. 397±34 mL/min). The results suggest that muscle contractions occurring during OP and that do not interfere with aBVS elicit a maximization of muscle functional hyperemia.

KEYWORDS: Muscle circulation, Vascular conductance, Electrical stimulation, synchronization

Introduction

The contraction of skeletal muscles increases both central hemodynamics as well as blood flow (BF) to the muscle territories engaged (10). It is commonly believed that exercise training has positive effects on the cardiovascular system, thus exerting a crucial preventive role against cardiovascular diseases (6, 23). It has been stated that such effects are mainly due to the increased pulse pressure and flow velocity wave amplitude elicited by exercise, involving the role of the endothelial release of Nitric Oxide (NO) (3, 16). Both acute and chronic effects have been documented: the former consisting in enhanced production of NO (and thus facilitated vasodilation) and the latter in remodeling of the small arteries and arterioles, with increased diameter and reduced wall thickness (7).

In as much as the above-mentioned effects of exercise on the cardiovascular system are caused by increased blood velocity and enhanced arterial pulsatility, there is still a contradiction. Specifically, skeletal muscle contraction per se opposes arterial pulsation and potentially impairs the free passage of blood from arterioles to venules due to the surge in intramuscular pressure during contractions. For instance, average femoral blood flow increases during alternate leg exercise, but instantaneous blood flow may be greatly reduced or even blocked altogether in certain phases of walking, running or even cycling (14, 20).

Some studies have focused on the physiological mechanisms necessary to achieve an optimal function of the heart and the contracting muscles (22, 24). With this purpose, O'Rourke et al. (22) studied this phenomena using an hydraulic-tubular-model in which 'muscle' contractions were synchronized to the 'heart' rate, suggesting that when the two pumps – representing left ventricular ejection and muscle contraction – were allowed to interact at nearly identical frequencies, the muscle perfusion would be maximized. In this hydraulic-tubular model, the optimal timing (late diastole/early systole, out of phase: OP) of muscle contraction and left ventricular ejection, showed an improvement in myocardial blood flow, coupled with lower systolic pressure.

77 On the other hand, when the timing of the two sine wave pumps was in-phase (IP), an increased
78 myocardial oxygen demand and attenuated left ventricular ejection occurred.

79 Indeed, several studies sought to translate such a model to humans, finding controversial
80 results (5, 12-14, 18, 29). Kirby et al. reported that cardiac rhythm was coupled with cycling at
81 specific intensities (13), however the results lacked a beat-by-beat analysis, which is crucial in
82 understanding this coupling phenomenon (14). Moreover, Kimura et al. (12) electrically
83 synchronized muscle contractions with heartbeats and demonstrated that the phase difference
84 between systole and the muscle contraction affects blood pressure (BP) and afterload during
85 intermittent electrical stimulation (ES). It is important to mention some limitations of the reported
86 literature; for instance, Kirby et al. assumed an exact coupling between systole and active muscle
87 contractions (13, 14), while Kimura et al. (12) set a standard pace for the electrically-evoked
88 contractions (20 Hz, 0.3 s of stimulation with a pause of 0.7 s) and categorized the heartbeats in
89 subgroups partitioned by given intervals between the pulses and the muscle contractions. As can be
90 seen in the first study, the synchronization was not finely controlled; whereas in the second case,
91 muscular pacing and heartbeats during the trials were taken at random, only on occasions when they
92 matched the muscle stimulation. In addition, the mechanical delay between ES and the appearance
93 of the muscle contraction is a key factor to achieve the perfect coupling with the blood pulse wave.
94 However, no previous studies have considered this factor, and a more rigorous approach is critical
95 for the analysis of this complicated physiological phenomenon.

96 Therefore, the purpose of this investigation was to study the peripheral and central
97 hemodynamic responses to a fine synchronization of electrically-evoked muscle contractions with
98 the heartbeat or, indeed, the blood velocity spectrum (BVS). We hypothesized that a muscle
99 contraction IP with the anterograde (or systolic) phase of BVS (aBVS) would impair BF and would
100 raise central hemodynamics levels; on the contrary, a muscle contraction OP with the aBVS, would
101 facilitate BF and reduce central hemodynamics.

Methods

Participants and General Procedures. Young and physically active males were recruited for this study. To be included, participants had to be in healthy condition without any known cardiovascular, neuromuscular, or metabolic diseases. All participants provided written informed consent before participation. The protocol (IRB#27111) is in accordance with the most recent revisions of the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Verona.

Experimental Design. Participants were positioned on a comfortable reclined chair with their back tilted 45° from the horizontal, and hip and knee at 85° for 10 minutes of rest and for the whole time of the study. The seat was positioned at a sufficient height to avoid any contact between the subject's feet and the floor. The protocol included two steps: 1) 5 minutes of electrically-evoked quadriceps isometric contractions synchronized in-phase (IP) with the aBVS in the femoral artery; 2) 5 minutes of electrically-evoked quadriceps isometric contractions synchronized out of phase (OP) with the aBVS in the femoral artery. The two conditions were performed in a random order, and full recovery from the previous test was monitored. Femoral blood flow (FBF), respiratory exchanges, central hemodynamics, mechanomyogram, electromyogram, and torque were recorded throughout the study.

Electrical Stimulation. After proper preparation, two full-surface solid adhesive hydrogel stimulating electrodes (size: 50x90 mm, Myotrode Plus, Globus G0465) were applied on the quadriceps: the anode was placed on the proximal part of the thigh, while the cathode was placed on the distal part of the leg extensors, 3 cm above the patella. Motor points were the location on the surface of the skin in which the electrical pulse evoked a tangible muscle twitch with the least amount of current. The stimulation intensity was determined before the measurements, with increments of 10 mA to a discomfort level of 3 (Tolerable) on a Visual Analogue Scale (VAS), and kept constant for the duration of the test. This, to avoid pain to the participants and sudden

uncontrolled increments of the heart rate elicited by noxious stimuli, which could have in turn increased the stimulation frequency. Trains of electrical stimuli (square-shaped, frequency: 20 Hz, pulse width: 260 μsec, duration: 200 msec) were delivered using a constant current stimulator (Digitimer DS7h, Welwyn Garden City, UK), interfaced with Powerlab (PowerLab 16/30; ML880; ADInstruments, Colorado Springs, CO), and controlled by a customized software. In detail, an Arduino microcontroller was programmed to obtain the trains of stimuli, verified by a Tektronix 100MHz oscilloscope.

Central hemodynamics. Resting BP was measured manually using a sphygmomanometer and a stethoscope placed over the brachial artery just below the cuff's edge. Throughout the trials, the arterial pressure waveform was continuously obtained with a plethysmograph device (Finapres®, FMS, Amsterdam, the Netherlands), and beat-to-beat systolic, diastolic, and mean arterial blood pressure (MAP) values were recorded. Measurements of heart rate (HR), stroke volume (SV), and cardiac output (CO) were collected using a cardioimpedence device (PhysioFlow®, Paris, France) for the first minute of rest and 5 minutes during the exercise. The skin was shaved and thoroughly cleaned before the application of electrocardiogram (ECG) electrodes.

Femoral Blood Flow. FBF of the exercising leg was measured at 1Hz resolution using a Doppler ultrasound (Logiq S7pro - General Electric Medical Systems, Milwaukee, WI) for the first minute of rest, and for 5 minutes during exercise. A 12-14 MHz probe was placed ~2-3 cm above the bifurcation of the common femoral artery. The blood velocity (V_{mean}) measurements were collected second-by-second using a pulsed mode with the probe placed at <60° angle, and the sample volume was centered and maximized to cover the vessel size. Measurements of the vessel diameter were obtained at rest in B-mode, with a 90° angle. Utilizing arterial diameter and V_{mean}, FBF flow was calculated as:

$$FBF = v_{mean} \cdot \pi \cdot \left(\frac{vessel\ diameter}{2}\right)^2 \cdot 60$$

where FBF is in milliliter per minute. Peripheral vascular conductance (PVC) was calculated using second by second recordings from the ultrasound and the mean arterial pressure from Finapress as: PVC = FBF x MAP^{-1}.

Respiratory variables. Ventilation (V_E) and pulmonary gas exchanges (VO_2, VCO_2) were measured breath-by-breath at rest and during the two trials using a metabolic cart ($QuarkB^2$; COSMED, Rome, Italy).

Torque. Torque was measured using a strain gauge (model UU2; DaCell, Korea) connected with a noncompliant strap at the stimulated limb ankle, then amplified and sampled at 1500 Hz using an external A/D converter. Torque is the product of force output and moment arm expressed in Newton-meters (Nm). The lever arm was measured from the noncompliant strap (2-3 cm above the lateral malleolus of the tibia) to the anatomical knee flexion-extension axis.

Mechanomyogram (MMG). A three-dimensional accelerometer (ADXL335, Adafruit Industries, New York, NY, USA) with a minimum scale range of ±3g was employed to detect the dimensional changes in activated motor units (9). The MMG sensor was applied over the highest point of the right rectus femoris muscle, in the area of maximal vertical displacement during contraction.

Electromyography (EMG). Although EMG is not an accurate assessment of the action potentials during ES, in the present study it was utilized to monitor the responses of the muscle throughout the trials. A two-electrode electromyograph (ZeroWire, Aurion, Italy) was placed on the vastus lateralis of the stimulated leg, approximately 10 cm above the head of the fibula, with a 20 mm inter-electrode distance, according to the procedure outlined by the SENIAM project (8).

Synchronization (Figure 1). On considering that our project aimed to obtain muscle contractions accurately timed with the systolic (IP) or the diastolic (OP) phases of femoral blood flow wave, we needed both the ECG signal to take advantage of a robust index of the beginning of the cardiac cycle, as well as an objective means to calculate proper delays. The delay with respect to the ECG R-wave accounts for the time of mechanical activation of the heart systole and the transmission speed of the sphygmic wave to the femoral artery. On the other hand, also the delay between the delivery of the stimulating train to the muscle and the mechanical muscle response shall be accounted for, in order to obtain the exact synchronization (either IP or OP) that we were seeking for. Three ECG electrodes were placed on the participant's chest and connected to the ADInstrument's Dual Bio Amp ECG/EKG analysis module (ML135) and to the ECG of the high-resolution ultrasound (Logiq S7pro - General Electric Medical Systems, Milwaukee, WI), to permit the acquisition of the same ECG and ensure the right synchronization of the signals. A mechanomyogram (MMG) and two stimulating electrodes connected to a constant current stimulator (Digitimer DS7h, Welwyn Garden City, UK) were interfaced with a Powerlab (PowerLab 16/30; ML880; ADInstruments, Colorado Springs, CO). All signals, excluding the blood flow acquired on the Ultrasound, were recorded simultaneously using LabChart v4.2 (ADInstruments, Colorado Springs, CO). For each participant, the time delay between the peak of the R-wave and the middle of the aBVS in the femoral artery was measured on the ultrasound, whereas the time delay between the onset of the ES and the tangible muscle contraction measured by the MMG was recorded on LabChart v4.2. A standard threshold was used to identify the peak of each R-wave on the ECG on LabChart; after the identification of the R-wave, a signal was automatically sent to an Arduino microcontroller, connected to the constant current stimulator, that delivered in due time a rectangular window of 200 ms to the constant current stimulator. Following this procedure, the stimulation was automatically delivered after every R-wave, with a customized delay for each participant and each protocol, allowing looped control of ECG and electrical stimulation of the muscle to manipulate contraction timing to the timing of the cardiac cycle and

thus the arrival of blood pulse to the leg; being either *in-phase* or *out of phase* with the stimulated contraction.

Data and Statistical analysis. A sample size of 10 participants was selected to ensure a statistical power higher than 0.80. Before analysis, all data were smoothed, using 12-s averages for individual baseline, and a 5-s rolling average for the 5 minutes of the trials. The data of the last minute of the recording were analyzed. All statistics were performed using SPSS version 23 (SPSS, Chicago, IL). Data were analyzed using a two-way repeated measure analysis of variance (ANOVA) to understand the impact of condition (in-phase vs. out of phase, 2 levels) and time (baseline and steady-state, 2 levels). Where ANOVA demonstrated a significant main effect, post-hoc analysis was conducted to identify differences between means using Tukey's HSD test. For all comparisons, statistical significance was declared when $P<0.05$. Data are presented as Means ± SD (IP vs. OP) throughout the paper and as Means ± SEM (IP vs. OP) in the figures.

Results

Study Participants and Baseline Values. Ten young, healthy, and physically active males were recruited. The mean age of the participants was 26 ± 3 years, the body weight was 76 ± 8 kg, and height was 179 ± 6 cm. The absolute resting levels of HR, SV, CO, MAP, FBF, and PVC were similar before the two trials ($P>0.05$, Table 1). Resting levels of V_E, VO_2, and VCO_2 were also similar at baseline ($P>0.05$).

Peripheral Hemodynamics (Figure 2). The effect of in-phase or out of phase synchronization was large and significant ($P<0.001$), as FBF was 48% greater in the OP (784 ± 25 mL/min) compared with the IP (454 ± 30 mL/min). In *Figure 1* the behavior of the FBF in the two conditions can be appreciated: in the IP (on the right), muscle contractions occurred during the BSV, likely impairing the free flow of blood, whereas in the OP (in the middle), muscle contractions were not interfering

with the BSV. Similarly, PVC rose significantly more (54%) during OP (6.7 ± 0.2 mL/min/mmHg), than during IP (4.3 ± 0.2 mL/min/mmHg, P<0.001). MAP attained a higher sustained value during the IP condition (113 ± 3 mmHg) in comparison to the OP (108 ± 2 mmHg, P<0.001).

Other Central Hemodynamics (Figures 3). The other hemodynamics variables were not affected differently by the two stimulation modalities, since no statistically significant differences (P>0.05) were observed between the two conditions. Specifically, during the out of phase steady-state HR (75 ± 11 bpm) showed similar values compared to IP (79 ± 10 bpm); accordingly, also SV (99 ± 28 mL) and CO (7.3 ± 1.6 L/min) were similar in the OP and in the IP (SV: 103 ± 25 mL; CO: 8.0 ± 1.6 L/min).

Respiratory responses (Figure 4). The respiratory responses were not different in the two conditions (P>0.05). No statistically significant differences were found in the gas exchange between IP (VO_2: 420.9 ± 69.9 mL/min; VCO_2: 365.7 ± 19.4 mL/min) and OP (VO_2: 397.4 ± 34.4 mL/min; VCO_2: 341.6 ± 25.2 mL/min) during steady-state. In accordance, the magnitude of the effect of the trials was equal, so that no statistical difference was observed in V_E between IP (14.2 ± 2.3 L/min) and OP (13.9 ±1.0 L/min).

Torque (Figure 5). As expected, since the stimulation intensity during the two trials was the same, the torque did not differ (IP: 5.6 ± 2.7 vs. OP: 5.8 ± 2.8 Nm, P>0.05). This lets us assert that the arterial inflow variations are an effect of the two conditions rather than of different metabolic demand of the contracting muscle.

Discussion

This study sought to parse the peripheral and central hemodynamic responses during the fine synchronization (systolic peak), or asynchronization (diastole), of electrically-evoked muscle contractions with the arrival of the BVS in the quadriceps. To control the synchronicity, the time delays between the ECG R-peak and the aBVS in the femoral artery, and the onset of ES and the

MMG peak, were calculated and utilized for triggering the ES cycle. Specifically, unilateral quadriceps contractions matched IP with the aBVS were compared to contractions matched OP with the aBVS. Our initial hypothesis was that a synchronization of the contraction OP with the aBVS would have enhanced the perfusion to the muscle, resulting in higher FBF. On the contrary, IP muscle contraction with the aBVS would have impaired FBF and elicited higher central hemodynamic responses. The findings of this study are mainly in accordance with our initial hypothesis, suggesting that muscle contractions occurring OP, therefore not interfering with aBVS, maximize muscle perfusion without impacting HR, SV, and CO.

Hemodynamic behavior during the synchronization of muscle contraction and aBVS: Different studies have explored the harmonization of muscle contraction with heartbeat (13, 14, 25, 29, 33). Walking (29), running (14, 25, 33), and cycling (13) were the most frequently performed tasks; however, the coupling phenomenon during a voluntary cyclic action is complicated and hard to synchronize. Considering this issue, the cited studies speculated how an optimized synchronization of the muscle contraction phase with the heartbeat could successfully result in an increased FBF to the muscle and lower cardiac afterload. Specifically, it has been reported that this type of matching method is correlated with lower values of SV (12), decreased PVR, and changes in pressure in the descending aorta due to vertical movement of the body during running (22). In our study, the design mostly averted the exercise-induced changes in BP by utilizing the ES technique. Despite the different approach, a similar HR and SV response occurred in both conditions, albeit elevated steady-state FBF and decreased MAP in the OP condition. Therefore, in accordance with the previous studies (14, 33), a muscle contraction that occurs OP with the arrival of the aBVS (or during the late diastole/early systole) maximizes the flow of blood to the muscle, and leads to a lowered systolic, diastolic BP, and afterload. The 48% increase in FBF during the OP in our results was not supported by a higher CO, but was accompanied by elevated PVC. Moreover, muscle

activity during the two conditions was equal, as confirmed by the ES intensity, torque, and whole-body VO_2. It has been shown that FBF through active muscles is cyclically occluded by rhythmic contractions, as a consequence of a rise in intramuscular pressure above systolic levels (30). In the present investigation, the elevated PVC that occurs during the late diastole/early systole (OP condition), reflects a facilitated or permissive mechanism for blood to reach and perfuse the active muscle. This greater FBF that occurs during the out of phase protocol leads to a probable increase in shear stress on the endothelium, which is considered the primary factor responsible for the beneficial vascular adaptations in humans (17, 26). When shear stress increases, it has been found that different factors are enhanced at the cellular level, such as arterial superoxide dismutase (SOD) I gene expression (27) and adenosine monophosphate-activated protein kinase (AMPK) (31), which in turn can likely result in greater NO bioavailability. It remains to be studied to which degree an ES training could influence these factors; however, it can be assumed that a synchronization that enhances blood supply to contracting muscle, can increase NO release, thus promoting arterial remodeling and minimizing skeletal muscle ischemia and/or cardiac energy cost.

Mechanisms of synchronization: Previous studies investigated how heartbeat could automatically synchronize to muscle contraction (4, 13, 14, 25). It has been previously suggested that cardiac rhythm may coordinate with muscle contractions in a rhythm-dependent manner (21), such that if the heartbeat occurs during lower intramuscular pressure in the active muscle, BF is likely augmented (19). Afferent signals from contracting muscles, whether voluntarily or electrically activated, may result in phase-dependent changes in the pacemaker cycle length affected by the autonomic nervous system (21). For instance, the timing of muscle contraction within the cardiac cycle influences the fluctuations of heartbeats (21). In our approach, electrically-evoked muscle contractions were triggered with customized delay by each cardiac cycle. A similar mechanism has been addressed only by a few other studies (12, 28). However, the differential effects on peripheral

circulation and on central hemodynamics were not adequately investigated. With the intention to finely synchronize muscle contractions with the heartbeat, two studies adapted ES to the cardiac cycle (12, 28). The stimulation trains (frequency: 20 Hz, pulse width: 260 μsec, duration: 200 msec) used in these previous studies were comparable to the current study, however the peripheral circulation was not investigated.

Practical applications: ES has been used to counteract neuromuscular disabilities, atrophy, muscular decline, and cardiovascular diseases in populations unable to perform voluntary exercise (1, 2, 11, 12, 32) due to the impaired mobility or forced immobilization. Previous studies observed how muscle ES in trains (20 Hz) of stimuli could provide the necessary increase in energy expenditure via repetitive muscle shortening (1, 15). The physiological responses to the stimulation-induced exercise were consistent with a light-to-moderate exercise intensity (1). Integrating these findings with our results suggests that trains of cyclic ES synchronized out of phase of the aBVS might be an interesting method by which to simulate light-moderate cardiovascular exercise (1), maximizing BF to contracting muscles, and thus shear stress on the vascular endothelium supplying the musculature. On recalling the positive effects of swinging shear stress due to the acceleration of blood in arteries at each heartbeat, we expect that proper synchronization of voluntary muscle contractions may also enhance the acute and chronic effects of exercise training. Moreover, the lower PVC and hence higher perfusion to contracting muscles during an OP stimulation permits an increased O_2 and nutrient delivery compared to a random ES. This method can be helpful to maximize the efficiency of a training session in people unable to exercise. Further, the occurrence of synchronicity might also help explain the optimized economy at certain cadences (e.g., cycling or running) in athletes at a given HR or intensity.

Study limitations: The intensity of ES in the present study was chosen with attention to the comfort of the participants, in order to avoid excessive pain, therefore the muscle contractions elicited must be considered equivalent to a very light exercise, albeit limited to a restricted group of muscles. As a consequence, no dramatic metabolic changes or force production could be expected. On the other hand, we aimed to finely check the timing of muscle contractions and to obtain an equal muscle involvement IP and OP. To appreciate the effects of the synchronization on general metabolism and muscle performance, either a stronger intensity or, if unfeasible, a more prolonged stimulation should be used. However, it has to be considered that the duration of the stimulation is limited by the available time between successive R waves.

Conclusion

This study documented that muscle contractions synchronized with the aBVS in the quadriceps can modulate blood flow to the active muscles during a period of 5 minutes. As previously suggested by an hydraulic-tubular model (22), a rhythmic muscle contraction coinciding with the aBVS implicates greater PVC, and FBF to the contracting muscle, with lower MAP and no changes of HR, SV, and CO. Therefore, it may be a winning strategy to maximize skeletal muscle perfusion in populations with severe exercise limitations. Future research should consider the potential effects of this approach investigating larger cohorts or more practical settings like voluntary locomotion.

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345

346 **Table 1. Absolute resting and Steady-State values of the participants**

Variable	IP		OP	
	Baseline	*Steady-State*	*Baseline*	*Steady-State*
HR (bpm)	69 ± 9	79 ± 10	66 ± 9	75 ± 11
SV (mL)	95 ± 21	103 ± 25	92 ± 22	99 ± 28
CO (L/min)	6.6 ± 1.4	8.0 ± 1.6	6.4 ± 1.3	7.3 ± 1.6
MAP (mmHg)	105.0 ± 2.8	113 ± 3	102.6 ± 1.3	108 ± 2 *
FBF (mL/min)	208 ± 102	454 ± 30	203 ± 93	784 ± 25 *
PVC (mL/min/mmHg)	2.5 ± 1.4	4.3 ± 0.2	3.4 ± 1.9	6.7 ± 0.2 *
Torque (Nm)	-	5.6 ± 2.7	-	5.8 ± 2.8

347

348 ***Note:*** *The absolute values are presented as Means* ± SD *(n=10). * = P<0.001 compared to IP*
349 *Steady-State. P>0.05 between OP and IP baseline conditions. HR = heart rate; SV = stroke volume;*
350 *CO = cardiac output; MAP = mean arterial pressure; FBF = femoral blood flow; PVC =*
351 *peripheral vascular conductance.*

352

353 *Figure legend*

354

355 ***Figure 1. Representative Baseline chart (left), synchro-time Out of Phase (middle) and In-Phase***
356 ***(right) on LabChart and Ultrasound views.*** *The chart displays the Finger Pulse (FP), Mean*
357 *Arterial Pressure (MAP), Electromyography (EMG), Torque, Mechanomyogram (MMG) and*
358 *electrical stimulation registered with Labchart; the femoral blood flow (BV) and the*
359 *electrocardiogram (ECG) registered on the ultrasound. All channels were recorded at the same*
360 *moment. The vertical bars correspond to the start and the end of the square-shaped train of*
361 *stimulation. In this view, we can appreciate the original non-filtered recordings and the delay*
362 *between the R-wave of the ECG and the stimulus in the two conditions with the different*
363 *correspondent BSV. The unfamiliar shape of the EMG is an artifact of the stimulation.*

364

365 ***Figure 2. Scatter Plot of Muscle Blood Flow during steady-state (n=10).*** *Relationship between A)*
366 *Femoral Blood Flow (FBF), B) Mean Arterial Pressure (MAP) and C) Vascular Conductance*
367 *(PVC) during the In-Phase (IP) and Out of Phase (OP) stimulations for individual pair*
368 *comparisons. The relationship shows higher FBF and PVC in the OP condition, with lower MAP*
369 *for all the participants. The dashed line represents the identity line; the white triangle represents*
370 *the Mean baseline of all participants; the black square represents the Mean result of all*
371 *participants. Values are presented as Means ± SEM;*

372

373 ***Figure 3. Scatter Plot of Central Hemodynamics during steady-state (n=10).*** *Relationship between*
374 *A) Stroke Volume (SV), B) Heart Rate (HR), and C) Cardiac Output (CO) during the In-Phase (IP)*
375 *and Out of Phase (OP) stimulations for individual pair comparisons. The dashed line represents the*

identity line; the white triangle represents the Mean baseline of all participants; the black square represents the Mean result of all participants. Values are presented as Means ± SEM.

Figure 4. Scatter Plot of Gas Exchanges during steady-state (n=10). *Relationship between A) Ventilation (VE), B) Volume of Oxygen Uptake (VO_2), and C) Volume of Carbon Dioxide (VCO_2) during the In-Phase (IP) and Out of Phase (OP) stimulations for individual pair comparisons. The dashed line represents the identity line; the white triangle represents the Mean baseline of all participants; the black square represents the Mean result of all participants. Values are presented as Means ± SEM.*

Figure 5. Scatter Plot of Torque during steady-state (n=10). *Relationship between the Torque measurements during the In-Phase (IP) and Out of Phase (OP) stimulations for individual pair comparisons. The dashed line represents the identity line; the white triangle represents the Mean baseline of all participants; the black square represents the Mean result of all participants. Values are presented as Means ± SEM.*

References

1. **Banerjee P, Clark A, Witte K, Crowe L, and Caulfield B**. Electrical stimulation of unloaded muscles causes cardiovascular exercise by increasing oxygen demand. *Eur J Cardiovasc Prev Rehabil* 12: 503-508, 2005.
2. **Barber L, Scicchitano BM, and Musaro A**. Molecular and Cellular Mechanisms of Muscle Aging and Sarcopenia and Effects of Electrical Stimulation in Seniors. *Eur J Transl Myol* 25: 231-236, 2015.
3. **Buckwalter JB, and Clifford PS**. Autonomic control of skeletal muscle blood flow at the onset of exercise. *Am J Physiol* 277: H1872-1877, 1999.
4. **Coleman W**. The psychological significance of bodily rhythms. *J Comp Psychol* 1: 213-220, 1921.

5. **Constantini K, Stickford ASL, Bleich JL, Mannheimer PD, Levine BD, and Chapman RF**. Synchronizing Gait with Cardiac Cycle Phase Alters Heart Rate Response during Running. *Med Sci Sports Exerc* 50: 1046-1053, 2018.
6. **Florido R, Kwak L, Lazo M, Nambi V, Ahmed HM, Hegde SM, Gerstenblith G, Blumenthal RS, Ballantyne CM, Selvin E, Folsom AR, Coresh J, and Ndumele CE**. Six-Year Changes in Physical Activity and the Risk of Incident Heart Failure: ARIC Study. *Circulation* 137: 2142-2151, 2018.
7. **Furchgott RF, and Zawadzki JV**. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288: 373-376, 1980.
8. **Hermens HJ, Freriks B, Disselhorst-Klug C, and Rau G**. Development of recommendations for SEMG sensors and sensor placement procedures. *J Electromyogr Kinesiol* 10: 361-374, 2000.
9. **Johnson MA, Polgar J, Weightman D, and Appleton D**. Data on the distribution of fibre types in 36 human muscles. *J Neurol Physiol* 18(1): 111-129, 1973.
10. **Joyner MJ, and Casey DP**. Regulation of increased blood flow (hyperemia) to muscles during exercise: a hierarchy of competing physiological needs. *Physiol Rev* 95: 549-601, 2015.
11. **Kern H, Barberi L, Lofler S, Sbardella S, Burggraf S, Fruhmann H, Carraro U, Mosole S, Sarabon N, Vogelauer M, Mayr W, Krenn M, Cvecka J, Romanello V, Pietrangelo L, Protasi F, Sandri M, Zampieri S, and Musaro A**. Electrical stimulation counteracts muscle decline in seniors. *Front Aging Neurosci* 6: 189, 2014.
12. **Kimura T, Kameda N, and Moritani T**. Impact of phase difference between cardiac systole and skeletal muscle contraction on hemodynamic response during electrically-induced muscle contractions. *J Electromyogr Kinesiol* 20: 572-579, 2010.
13. **Kirby RL, MacLeod DA, and Marble AE**. Coupling between cardiac and locomotor rhythms: the phase lag between heart beats and pedal thrusts. *Angiology* 40: 620-625, 1989.
14. **Kirby RL, Nugent ST, Marlow RW, MacLeod DA, and Marble AE**. Coupling of cardiac and locomotor rhythms. *J Appl Physiol (1985)* 66: 323-329, 1989.
15. **McArdle W, Katch F, and Katch V**. *Exercise physiology: energy, nutrition, and human performance.* London: Lippincott Williams and Wilkins, 2001.
16. **McCloskey DI, and Mitchell JH**. Reflex cardiovascular and respiratory responses originating in exercising muscle. *J Physiol* 224: 173-186, 1972.
17. **Morishima T, Restaino RM, Walsh LK, Kanaley JA, Fadel PJ, and Padilla J**. Prolonged sitting-induced leg endothelial dysfunction is prevented by fidgeting. *Am J Physiol Heart Circ Physiol* 311: H177-182, 2016.
18. **Niizeki K**. Heart abhors contracting during elevated intramuscular pressure: implication for cardiac-locomotor synchronization. In: *SICE 2004 Annual Conference*. Sapporo, Japan: 2004, p. 2019-2112.
19. **Niizeki K**. Intramuscular pressure-induced inhibition of cardiac contraction: implications for cardiac-locomotor synchronization. *Am J Physiol Regul Integr Comp Physiol* 288: R645-650, 2005.
20. **Niizeki K, Kawahara K, and Miyamoto Y**. Interaction among cardiac, respiratory, and locomotor rhythms during cardiolocomotor synchronization. *J Appl Physiol (1985)* 75: 1815-1821, 1993.
21. **Niizeki K, and Miyamoto Y**. Phase-dependent heartbeat modulation by muscle contractions during dynamic handgrip in humans. *Am J Physiol* 276: H1331-1338, 1999.
22. **O'Rourke M, and Avolio A**. Improved cardiovascular performance with optimal entertainment between heart rate and step rate during running in humans. *Coronary Artery Disease* 3: 863-869, 1992.
23. **Paffenbarger RS, Jr., Hyde RT, Wing AL, and Hsieh CC**. Physical activity, all-cause mortality, and longevity of college alumni. *N Engl J Med* 314: 605-613, 1986.
24. **Palatini P, Mos L, Mormino P, Di Marco A, Munari L, Fazio G, Giuliano G, Pessina AC, and Dal Palu C**. Blood pressure changes during running in humans: the "beat" phenomenon. *J Appl Physiol (1985)* 67: 52-59, 1989.
25. **Phillips B, and Jin Y**. Effect of adaptive paced cardiolocomotor synchronization during running: a preliminary study. *J Sports Sci Med* 12: 381-387, 2013.
26. **Restaino RM, Walsh LK, Morishima T, Vranish JR, Martinez-Lemus LA, Fadel PJ, and Padilla J**. Endothelial dysfunction following prolonged sitting is mediated by a reduction in shear stress. *Am J Physiol Heart Circ Physiol* 310: H648-653, 2016.
27. **Rush JW, Laughlin MH, Woodman CR, and Price EM**. SOD-1 expression in pig coronary arterioles is increased by exercise training. *Am J Physiol Heart Circ Physiol* 279: H2068-2076, 2000.
28. **Sasaki KI, Matsuse H, Akimoto R, Kamiya S, Moritani T, Sasaki M, Ishizaki Y, Ohtsuka M, Nakayoshi T, Ueno T, Shiba N, and Fukumoto Y**. Cardiac cycle-synchronized electrical muscle stimulator

459 for lower limb training with the potential to reduce the heart's pumping workload. *PLoS One* 12: e0187395,
460 2017.
461 29. **Takeuchi S, Nishida Y, and Mizushima T**. Effects of Synchronization between Cardiac and
462 Locomotor Rhythms on Oxygen Pulse during Walking. *Journal of Sports Science and Medicine* 13: 881-887,
463 2014.
464 30. **Walloe L, and Wesche J**. Time course and magnitude of blood flow changes in the human
465 quadriceps muscles during and following rhythmic exercise. *J Physiol* 405: 257-273, 1988.
466 31. **Wang X, Wang Q, and Sun Z**. Normal IgG downregulates the intracellular superoxide level and
467 attenuates migration and permeability in human aortic endothelial cells isolated from a hypertensive patient.
468 *Hypertension* 60: 818-826, 2012.
469 32. **Zampieri S, Mosole S, Lofler S, Fruhmann H, Burggraf S, Cvecka J, Hamar D, Sedliak M,**
470 **Tirptakova V, Sarabon N, Mayr W, and Kern H**. Physical Exercise in Aging: Nine Weeks of Leg Press or
471 Electrical Stimulation Training in 70 Years Old Sedentary Elderly People. *Eur J Transl Myol* 25: 237-242,
472 2015.
473 33. **Zhang D, Celler BG, and Lovell NH**. The effect of heartbeat-synchronised running on the
474 cardiovascular system. In: *Proceedings of the Second Joint 24th Annual Conference and the Annual Fall*
475 *Meeting of the Biomedical Engineering Society] [Engineering in Medicine and Biology*2002, p. 1295-1296.

476

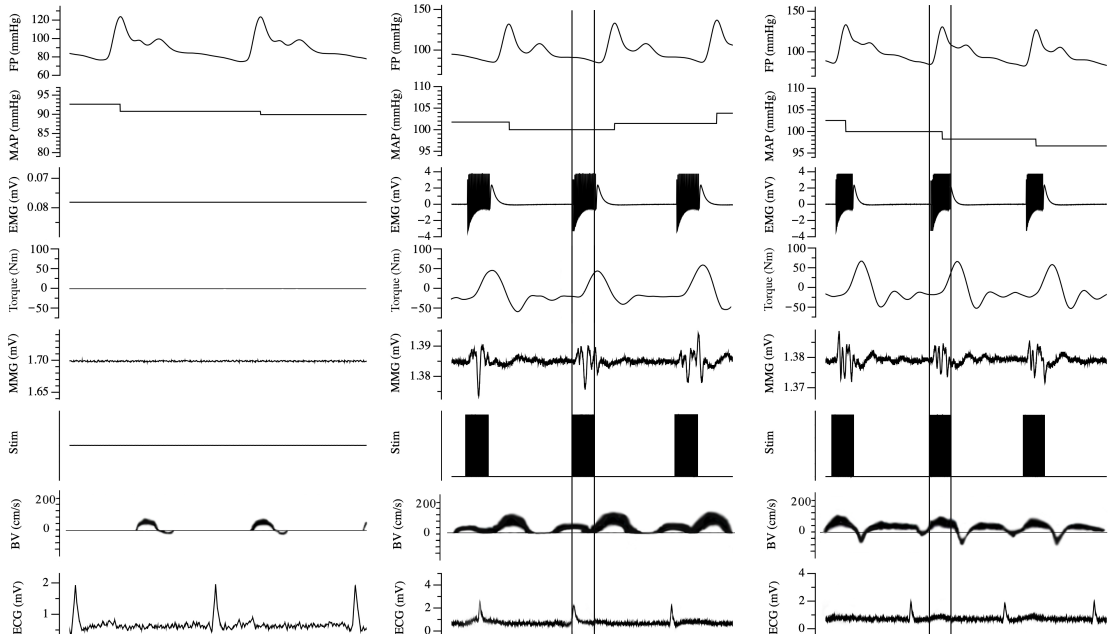
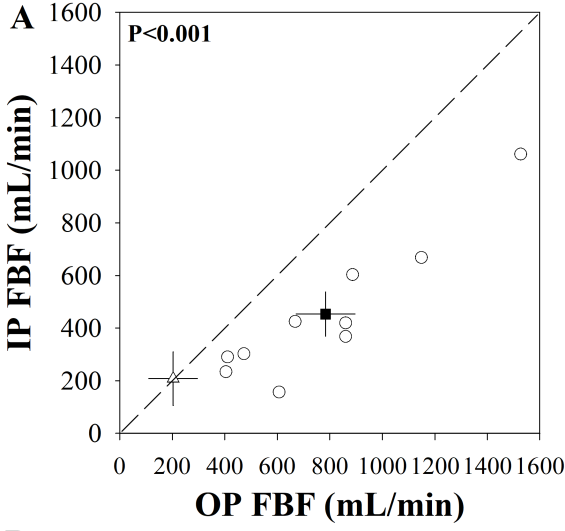
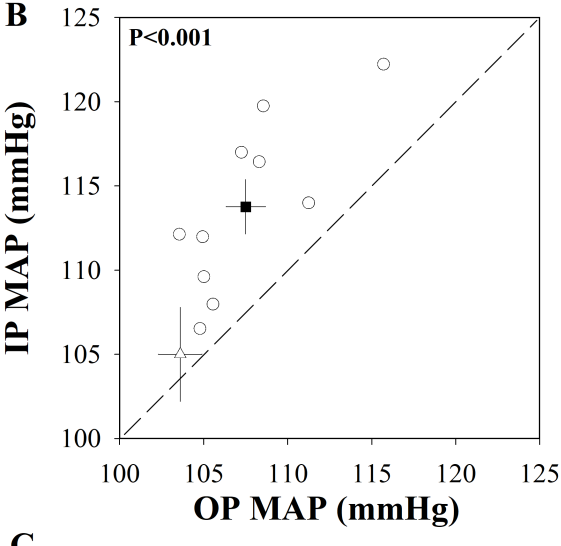


Figure 2





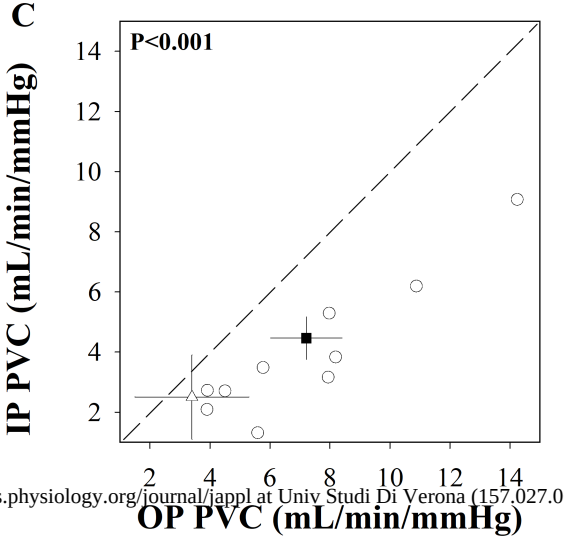


Figure 3

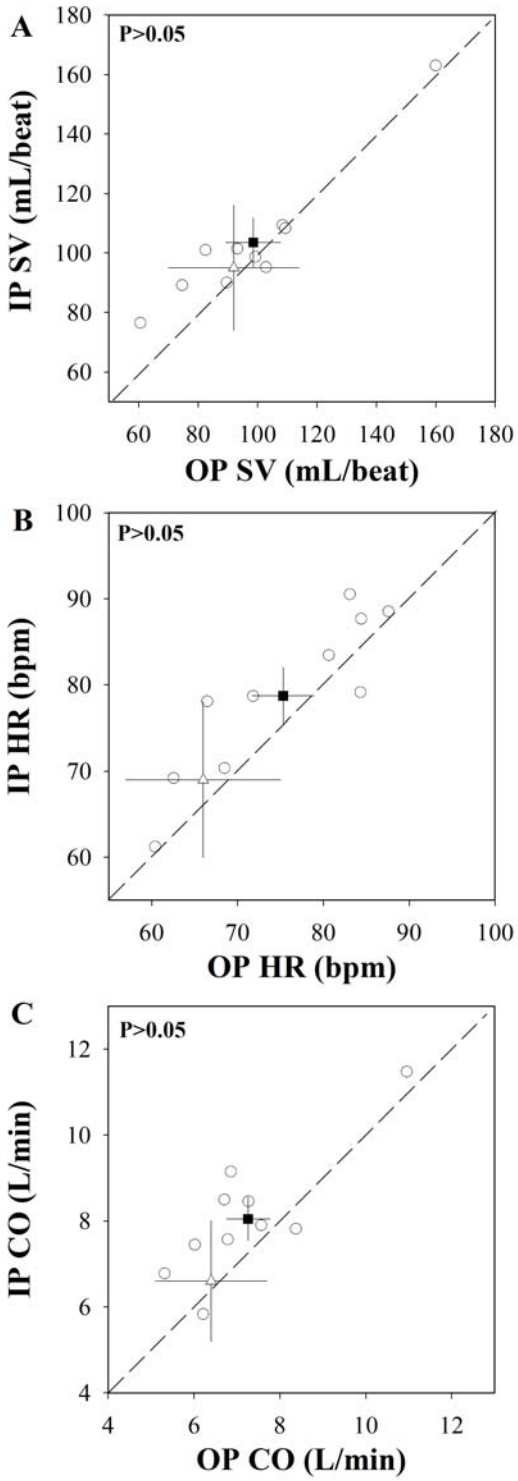


Figure 4

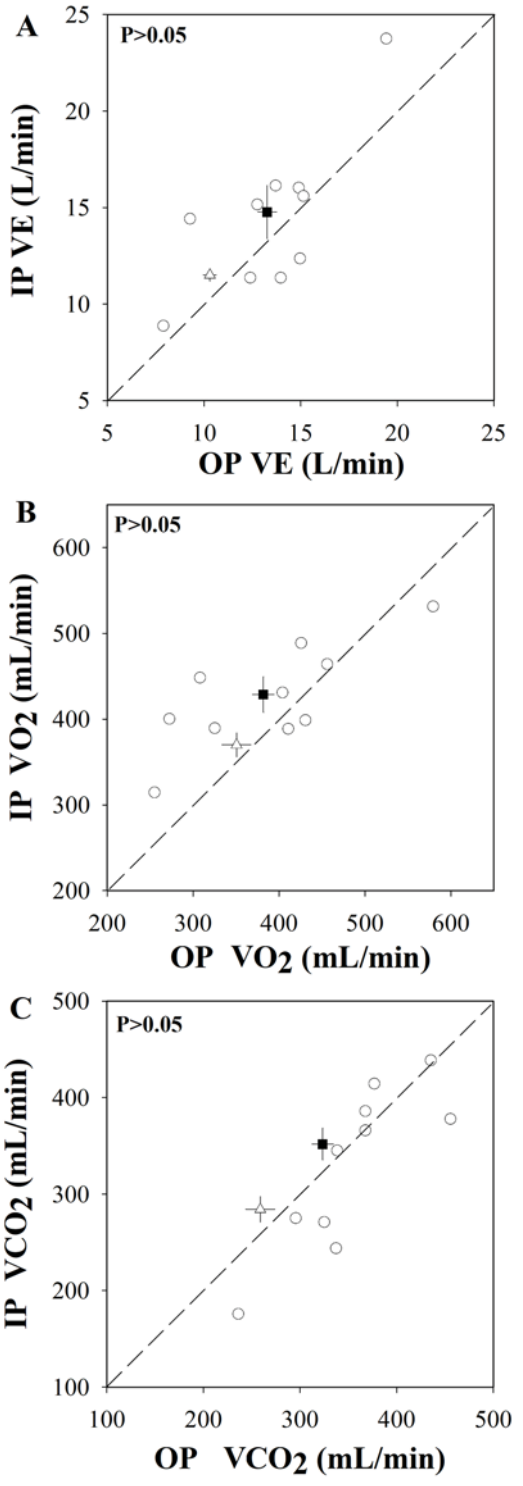


Figure 5