Published online: 2020-01-20

Effect of Lower Body Negative Pressure on Phase I Cardiovascular Responses at Exercise Onset

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Authors

Nazzareno Fagoni[1], Paolo Bruseghini[1], Alessandra Adami[2], Carlo Capelli[3], Frederic Lador[4], Christian Moia[5], Enrico Tam[6], Aurélien Bringard[7], Guido Ferretti[5]

Affiliations

1 Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy
2 Department of Kinesiology, University of Rhode Island, United States
3 Department of Physical Performances, Norwegian School of Sport Sciences, Oslo, Norway
4 Division de Pneumologie, Département des Spécialités de Médecine, Hôpitaux Universitaires de Genève, Geneva, Switzerland
5 Département des Neurosciences Fondamentales, Université de Genève Centre Médical Universitaire, Geneve, Switzerland
6 Dipartimento di Scienze Neurologiche e della Visione, University of Verona, Verona, Italy
7 University Medical Center, Basic Neurosciences, Geneva, Switzerland

Key words

cardiac output, stroke volume, heart rate, oxygen uptake, kinetics and exercise transient, LBNP

accepted 30.09.2019

Bibliography

DOI https://doi.org/10.1055/a-1028-7496
Published online: 20.1.2020
Int J Sports Med 2020; 41: 209–218

ISSN 0172-4622

Correspondence

Nazzareno Fagoni, MD, PhD
Department of Molecular and Translational Medicine
University of Brescia
Viale Europa, 11
25123 Brescia
Italy
Tel : + 393493586821, Fax : + 390303995779
nazzarenofagoni@gmail.com

ABSTRACT

We hypothesised that vagal withdrawal and increased venous return interact in determining the rapid cardiac output (CO) response (phase I) at exercise onset. We used lower body negative pressure (LBNP) to increase blood distribution to the heart by muscle pump action and reduce resting vagal activity. We expected a larger increase in stroke volume (SV) and smaller for heart rate (HR) at progressively stronger LBNP levels, therefore CO response would remain unchanged. To this aim ten young, healthy males performed a 50 W exercise in supine position at 0 (Control), − 15, − 30 and − 45 mmHg LBNP exposure. On single beat basis, we measured HR, SV, and CO. Oxygen uptake was measured breath-by-breath. Phase I response amplitudes were obtained applying an exponential model. LBNP increased SV response amplitude threefold from Control to − 45 mmHg. HR response amplitude tended to decrease and prevented changes in CO response. The rapid response of CO explained that of oxygen uptake. The rapid SV kinetics at exercise onset is compatible with an increased venous return, whereas the vagal withdrawal conjecture cannot be dismissed for HR. The rapid CO response may indeed be the result of two independent yet parallel mechanisms, one acting on SV, the other on HR.

Introduction

Whipp et al. [1] were the first to propose that the kinetics of pulmonary oxygen uptake ($\dot{V}O_2$) at exercise onset involves two distinct phases. They attributed the rapid $\dot{V}O_2$ increase (Phase I, φ1), originally identified by Wasserman et al. to a sudden increase in cardiac output (CO) (cardiodynamic phase) [2]. The subsequent slower phase (Phase II, φ2) was associated with muscle metabolic adaptations [3–6]. Based on these premises, Barstow and Molé [7] developed a double-exponential model for analyzing the $\dot{V}O_2$ kinetics. φ1 interpretation received experimental support when beat-by-beat methods for CO determination became available, allowing an analysis of CO kinetics during exercise transients [8–10]. So, application of the model by Barstow and Molé [7] to the kinetics of both $\dot{V}O_2$ and CO demonstrated very fast time constants for φ1 (τ_1), of the order of 1-to - 3 s. Subsequent studies have shown that the φ1 amplitude (A_1) of $\dot{V}O_2$ was explained by the sudden CO increase at exercise onset during φ1, since the entity of A_1 of CO and $\dot{V}O_2$ was the same [11, 12].

Lador et al. [11] attributed the φ1 of CO to sudden withdrawal of vagal tone at exercise start (vagal withdrawal conjecture), on the basis of previous experiments on heart rate (HR) kinetics under vagal blockade [13].

If the vagal withdrawal conjecture was correct, we would expect greater A_1 of HR and A_1 of CO in presence of strong vagal tone. For instance, vagal activation at rest is greater in supine than in upright position [14, 15], and less intense in acute hypoxia than in normoxia [16, 17]. In fact, A_1 of CO, although smaller in hypoxia than in normoxia, is only partially explained by A_1 of HR [12]. On the other hand, some studies suggested a possible lack of φ1 for CO in supine posture [18], contrary to expectations.

Therefore, another mechanism could act in parallel to the autonomic nervous system modulation. Indirect evidence in humans suggests that, similar to animals [19], a sudden increment in venous return may induce a rapid stroke volume (SV) increase at the onset of exercise (heart filling conjecture) [15, 18, 20–22]. Also Nobrega et al. postulated similar mechanisms to explain the CO increase during light isometric exercise in patients carrying pacemakers for complete heart block [23]. The SV increase is due to blood displacement from the lower limbs by muscle pump action, and contributes to the rapid CO increase in φ1. This mechanism could explain the results of φ1 of CO, which can hardly be reconciled with the vagal withdrawal conjecture. These two conjectures may not be mutually exclusive, but may coexist and act simultaneously in determining the apparent CO response in φ1.

Lower body negative pressure (LBNP) has been used as a tool to displace blood toward the lower limbs in a controlled manner [24, 25]. The stronger the LBNP level, the greater the amount of blood displaced toward the heart at exercise onset. According to the heart filling conjecture, a higher SV response should result in a higher A_1 of CO. However, the LBNP model also induces a parallel reduction of the resting vagal activity [26]. So, we should expect at least a decrease in A_1 of HR, tending to lower the A_1 of CO. Therefore, LBNP is a crucial tool for testing these two conjectures and discerning their respective roles in the φ1 kinetics of CO. Nevertheless, no investigation of the effects of increasing absolute levels of LBNP exposure on the φ1 dynamics of SV and CO was carried out so far, to the best of our knowledge.

The aim of this study was to investigate the changes in φ1 kinetics of CO, SV and HR during constant-load aerobic exercise, performed in supine posture and to progressive increase in LBNP level. We hypothesized that, as the LBNP level is increased, 1) the A_1 of SV should become progressively higher, 2) this effect would be counteracted by a lower A_1 of HR, and 3) the φ1 time constant would remain unchanged. The amplitudes of SV and HR will determine the A_1 of CO. This would explain the persistence of a φ1 of CO in conditions characterized by reduced vagal activity at rest and suggest why performing aerobic exercise in conditions such as ageing, hypoxia and disuse should be a safe practice.

Materials and Methods

Participants

Ten healthy non-smoking individuals took part in the experiments. They were (mean ± SD) 24.8 ± 4.7 years old, 174 ± 7 cm tall, and 71.7 ± 12.5 kg body mass. All subjects were informed of procedures and risks associated with the experimental design, the right to withdraw from the study at any time without jeopardy, and signed an informed consent form. The study was conducted in accordance with the Declaration of Helsinki. The ethical committee of Geneva University Hospital approved the protocol. The authors declare that this study meets the ethical standards of the journal [27].

Measurements

HR was continuously measured by electrocardiography (Elmed ETM 2000, Heiligenhaus, Germany). Continuous recordings of arterial pulse pressure profile were obtained at a fingertip of the left hand by means of a non-invasive cuff pressure recorder (Portapres, FMS, Amsterdam, the Netherlands). Beat-by-beat mean arterial pressure (MAP) was computed as the integral mean of each pressure profile, using the Beatscope® software package (FMS, Amsterdam, the Netherlands). To check the reliability of the values provided by the Portapres, blood pressure was also measured at steady state, using a sphygmomanometer placed on the contralateral arm. A difference between Portapres and the sphygmomanometer greater than 10 mmHg in MAP led to interrupt the experiment, to calibrate again the instrumentation and to repeat the protocol.

Single beat SV was determined by means of the Modelflow method [28], applied off-line to the pulse pressure profiles, using the Beatscope® software package. Beat-by-beat CO was computed as the product of single beat SV times the corresponding single beat HR. Steady state CO values were also obtained at min 5 by means of the open circuit acetylene method [29]. This method implies breathing a normoxic gas mixture containing 1 % acetylene and 5 % helium for at least 20 respiratory cycles [11, 29]. The partition coefficient for acetylene was determined for each participant by mass spectrometry on 5-ml venous blood samples. Individual correction factors were calculated by dividing the CO values obtained with the acetylene technique by the corresponding CO values obtained with the Modelflow method. The data were then corrected for the method's inaccuracy, as previously described [11, 30, 31].

$\dot{V}O_2$ was determined on a breath-by-breath basis. The time course of oxygen and carbon dioxide partial pressures throughout the respiratory cycles were continuously monitored by a mass spectrometer (Balzers Prisma, Balzers, Liechtenstein) calibrated against gas mixtures of known composition. The inspiratory and expiratory ventilations were measured by an ultrasonic flowmeter (Spiroson, Ecomedics, Duernten, Switzerland) calibrated with a 3 l syringe. The traces alignment was corrected off-line for the time delay between the flowmeter and the mass spectrometer. Breath-by-breath $\dot{V}O_2$ and carbon dioxide output ($\dot{V}CO_2$) were then computed off-line by means of a modified version of the Grønlund's algorithm [32]. Software purposely written under the Labview® developing environment (Labview® 5.0, National Instruments, Austin, TX, USA) was used for this purpose. The characteristics and the physiological implications of the Grønlund's algorithm were widely discussed elsewhere [33, 34].

All the signals were digitized in parallel by a 16-channel A/D converter (MP150, Biopac Systems, Goleta CA, USA). The acquisition rate was set at 200 Hz.

Blood lactate concentration ($[La]_b$) was measured by an electro enzymatic method (Eppendorf EBIO 6666, Erlangen, Germany) on 10 µl blood samples taken from the left earlobe. Arterialized blood gas composition was measured by microelectrodes (Instrumentation Laboratory Synthesis 10, Lexington, MA, USA) on 50 µl blood samples taken from the right earlobe.

The LBNP device

The LBNP device was a wooden box 121 cm long, 73 cm tall and 63 cm large (walls 3 cm thick), mounted on a 220 cm bed, where participants lay supine, with the lower part, i.e. from the belly to the feet, in the box. When negative pressure was applied, the inner box was isolated from the external ambient by means of a neoprene kayak skirt. On one side, the skirt was fixed to the box opening, on the other it was tightly tied to the belly by a system of belts, so that no air inlet was allowed through the skirt. Negative pressure inside the box was generated by a vacuum cleaner. The air outflow through the vacuum cleaner was regulated by an adjustable leak, until the pre-selected negative pressure was obtained. The pressure inside the box was continuously controlled by means of a manometer.

An electrically braked cycle ergometer (Ergoselect 400, Ergoline GmbH, Bitz, Germany), modified for leg pedaling in supine posture, was mounted on a rail placed on the inner bottom of the LBNP box. Race cycling shoes and pedals were used to facilitate exercise performance while the negative pressure was applied, as well as to help the subjects to stay motionless and relaxed during rest periods. The electro-mechanical characteristics of the ergometer were such as to permit workload application in less than 50 ms.

Protocol

The participant lay supine on a bed, where the ergometer and LBNP device were mounted. After instrumentation, the protocol started with arterialized blood sampling and the assessment of acetylene CO at rest, followed by 3 min of monitoring in quiet rest.

The participant performed a series of three exercises at 50 W, starting from rest. The first exercise lasted between 6 and 7 min, the following exercises were 5 min long. Each subject maintained the same pedaling frequency throughout the entire series of experiments (visual feedback), between 60 and 80 rpm. The pedaling frequency was recorded for off-line detection of the time of the actual exercise start. For all exercises, arterialized blood gas composition and $[La]_b$ were measured at min 5. In the first exercise, after these measurements, we determined CO by the open circuit acetylene method. Each exercise was followed by 6-min recovery, during which $[La]_b$ was measured at min 1, 3 and 5 and arterialized blood gas composition at min 5.

Although this paper is devoted to φ1 analysis only, the 5-minutes exercise duration was chosen in order to: 1) attain a steady state, allowing appropriate application of the double exponential model to data analysis and obtaining steady state data for measurement quality control; and 2) calibrate the single beat data of SV and CO against an established steady state method. Individual correction factors for beat-by-beat CO values were calculated using the acetylene CO values as reference, and applied during dynamic states with rapid changes in CO [35]. The calibration factors were unaffected by the LBNP level, whether at rest (0.97 ± 0.32, 1.15 ± 0.10, 1.06 ± 0.27 and 0.93 ± 0.26 in Control, −15, −30 and −45 mmHg, respectively, NS) or during exercise (1.03 ± 0.20, 0.95 ± 0.20, 0.98 ± 0.27 and 0.97 ± 0.15 in Control, −15, −30 and −45 mmHg, respectively, NS).

Each participant repeated the experimental protocol in four conditions, administered in random order: Control, i.e. without negative pressure (LBNP box open, vacuum cleaner turned off); and three LBNP levels, at −15, −30 and −45 mmHg with respect to ambient pressure. Under LBNP, a further 3-min rest was applied to allow the participant adapting to the pressure.

Data treatment

Total peripheral resistance (TPR) was calculated by dividing each MAP value by the corresponding calibrated CO value, on the assumption that the pressure in the right atrium can be neglected as a determinant of peripheral resistance [11]. Steady state data for each variable were then obtained as the mean of the beat-by-beat values at rest, between 70 and 10 s before exercise start, and during the 5th min of exercise. The arterial-venous oxygen concentration difference (Ca_{O_2}-$C\bar{v}_{O_2}$) was calculated at rest and during exercise steady state, as the ratio between steady state $\dot{V}O_2$ and CO, as discussed elsewhere [36, 37].

For each subject, the breath-by-breath $\dot{V}O_2$ values from the three repetitions were time aligned and superimposed to obtain a single trace for each condition [38]. The same approach was used for analyses of HR, SV and CO on a beat-by-beat basis. On the superimposed time series of single-beat or single-breath values for each variable, a double-exponential model of exercise transients, with positive time delay for φ2, was used to fit the signal (Equation 1) [7, 11].

$$[\dot{V}O_2 \text{ or HR or SV or CO}](t) = A_1 (1 - e^{-k1}) + H(t-d)\, A_2 (1 - e^{-k2(t-d)}) \quad (1)$$

where k_1 and k_2 are the velocity constants of the exponential parameters increase in φ1 and φ2, respectively, d is the time delay, and A_1 and A_2 are the amplitude of the parameter increase during

φ1 and φ2. H(t-d) is the Heaviside function defined as 0 if t < d and 1 if t ≥ d.

Although the fitting method analyzed both φ1 and φ2, only results concerning φ1 will be reported and discussed from now on.

We also analyzed the quantitative compatibility between the A_1 of CO and that of $\dot{V}O_2$, computing $\Delta\dot{V}O_2$ by means of the following equation:

$$\dot{V}O_{2r} + \Delta\dot{V}O_2 = (CO_r + \Delta CO) * (Ca_{O_2} - C\bar{v}_{O_2}) \quad (2)$$

Because of a delay between muscle O_2 consumption and $\dot{V}O_2$, we can assume that $Ca_{O_2} - C\bar{v}_{O_2}$ remains unchanged during φ1 [7, 11]. On this basis, the Fick principle allows a prediction of the expected $\dot{V}O_2$ increase in φ1 as a consequence of CO increase. Individual resting values for $Ca_{O_2} - C\bar{v}_{O_2}$ were used for calculation of the $\Delta\dot{V}O_2$ by means of equation 2, where $\dot{V}O_{2r}$ is the resting $\dot{V}O_2$, whereas $\Delta\dot{V}O_2$ is the corresponding increment during φ1.

Statistics

Data are given as mean and standard deviation (SD). The effects of LBNP on the parameters of the kinetics for HR, SV, CO and $\dot{V}O_2$ were analysed by one-way analysis of variance for repeated measures. The effects of the experimental conditions at steady state (rest vs. exercise, and Control vs. −15, −30 and −45 mmHg LBNP levels) were analyzed by two-way analysis of variance for repeated measures. When applicable, the LSD Fischer post-hoc test was used to locate significant differences. The results were considered significant if $p < 0.05$. The parameters of the double-exponential model were estimated by utilizing a weighted non-linear least squares procedure [39], implemented in Matlab (version 7.9.0, MathWorks, Natick, MA, USA). One sample student t-test was used to compare measured and calculated data between ▶ **Tables 1** and ▶ **2**.

Results

An example of the time courses of measured or calculated variables upon the onset of exercise appears in ▶ **Fig. 1**, for Control and −45 mmHg LBNP. A zoom of ▶ **Fig. 1** on an exercise time basis of 20 s, thus evidencing only φ1, is reported in ▶ **Fig. 2**. As exercise started, HR, SV, CO and $\dot{V}O_2$ increased, whereas TPR decreased, to reach a new steady state. MAP underwent an initial rapid drop, the amplitude of which was between 5 and 10 mmHg, then increased slowly and progressively toward a new steady state.

Transients

The parameters of the double-exponential equations describing the HR, SV, CO and $\dot{V}O_2$ kinetics in φ1 are shown in ▶ **Table 1**.

SV A_1 was higher under LBNP than in Control ($p < 0.05$ in −45 mmHg and −30 mmHg LBNP as compared to Control).

Conversely, the A_1 of HR did not change between −15 mmHg LBNP and Control (NS), and tended to drop only when moving from −15 to −30 and −45 mmHg LBNP (NS). At −45 mmHg, it was 23 ± 56 % lower than in Control (NS).

Therefore, the A_1 of CO was unaffected by LBNP, although the value at −15 mmHg LBNP was 0.8 L min^{-1} higher than in Control (NS), entirely due to the increase in A_1 of SV.

$\dot{V}O_2$ A_1 was higher at −45 mmHg LBNP than in Control ($p < 0.05$).

A theoretical computation of the parameters of the φ1 kinetics of CO (▶ **Table 2**) was carried out by multiplying the exponential equations describing the kinetics of HR and SV. To this aim we used the mean values of the studied group, for Control and the different LBNP levels. These values have been compared to the A_1 of CO of ▶ **Table 1**.

The results of this further analysis show that the mean τ_1 and A_1 for CO, reported in ▶ **Table 1**, did not differ from the theoretical parameters of ▶ **Table 2**.

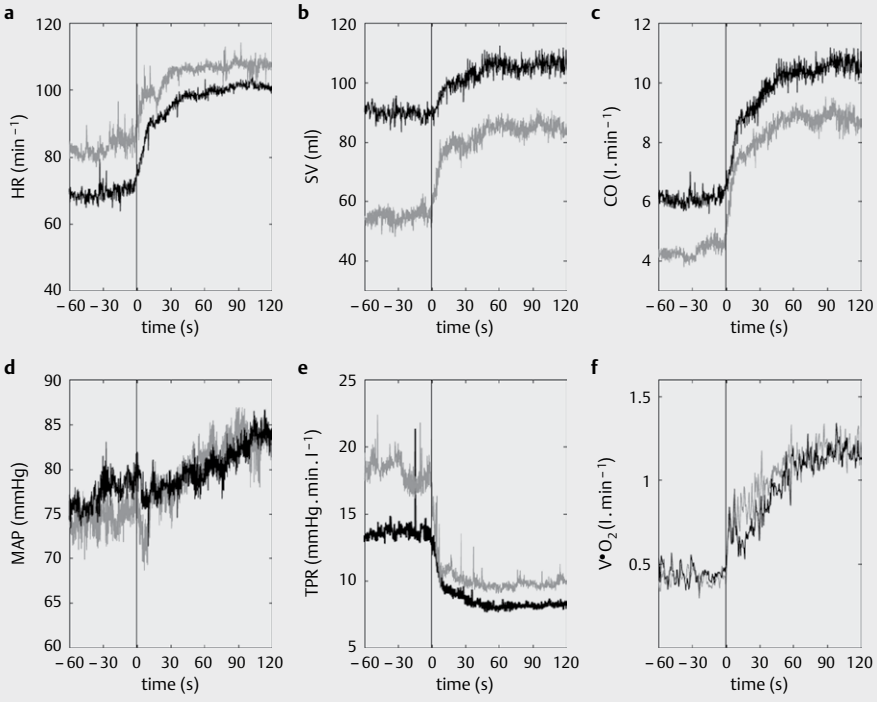
▶ **Table 1** Phase 1 amplitudes (A_1) and time constants (τ_1) for heart rate (HR), stroke volume (SV), cardiac output (CO) and oxygen uptake ($\dot{V}O_2$) at exercise onset, during Control and the LBNP experimental conditions.

		Control	−15 mmHg	−30 mmHg	−45 mmHg
HR	A_1 (min^{-1})	19 ± 7	20 ± 6	18 ± 6	14 ± 10
	τ_1 (s)	3.28 ± 1.92	3.00 ± 1.26	1.87 ± 1.62	2.42 ± 1.39
SV	A_1 (ml)	6.8 ± 3.5	9.9 ± 4.6	14.6 ± 7.3 *	19.8 ± 10.0 * $
	τ_1 (s)	2.31 ± 1.31	1.79 ± 2.04	2.05 ± 2.00	2.82 ± 2.65
CO	A_1 (l min^{-1})	1.83 ± 1.01	2.62 ± 1.17	2.87 ± 0.91	2.85 ± 1.28
	τ_1 (s)	3.70 ± 2.77	3.65 ± 1.67	3.12 ± 1.58	2.24 ± 1.33
$\dot{V}O_2$	A_1 (l min^{-1})	0.22 ± 0.12	0.23 ± 0.08	0.26 ± 0.12	0.35 ± 0.15 *
	τ_1 (s)	0.97 ± 1.88	0.24 ± 0.08	0.90 ± 1.07	1.54 ± 1.76

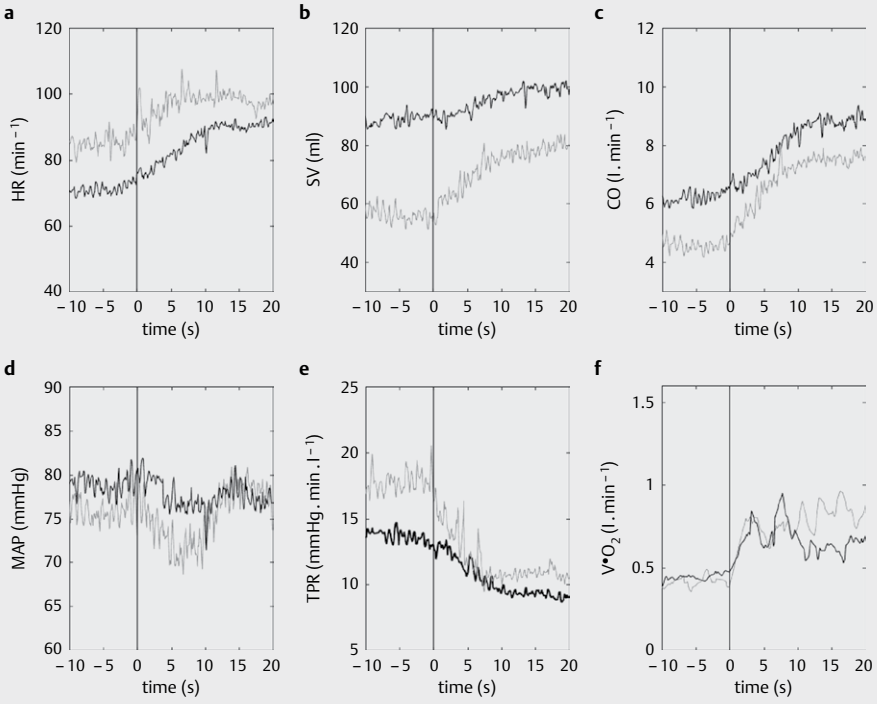
Values are given as means ± SD. * = significantly different from Control ($p < 0.05$), $ = significantly different from −15 mmHg ($p < 0.05$).

▶ **Table 2** Amplitude (A_1) and time constant (τ_1) of the phase I (φ1) kinetics of cardiac output (CO), obtained by multiplying the two exponential equations describing the φ1 kinetics for stroke volume and heart rate (NS compared to measured data reported in ▶ **Table 1**).

Cardiac Output (CO)	Control	−15 mmHg	−30 mmHg	−45 mmHg
A_1 (l min^{-1})	2.21	2.55	2.53	2.83
τ_1 (s)	3.25	2.98	2.01	2.79



▶ **Fig. 1** Example of the time course of beat-by-beat heart rate (HR, panel **a**), stroke volume (SV, panel **b**), cardiac output (CO, panel **c**), mean arterial pressure (MAP, panel **d**), total peripheral resistance (TPR, panel **e**) and oxygen uptake ($\dot{V}O_2$, panel **f**) upon the onset of exercise, for Control (black line) and −45 mmHg LBNP (grey line). Vertical lines at time 0 correspond to the start of exercise.



▶ **Fig. 2** Representation of the first 20 seconds at exercise onset (φ1, zoom of ▶ **Fig. 1**).

Steady state

The steady state data obtained in Control at rest and during exercise are reported in ▶ **Table 3**.

The effects of LBNP exposure appear in ▶ **Fig. 3**. Steady state $\dot{V}O_2$ was unaffected by LBNP exposure (▶ **Fig. 3f**).

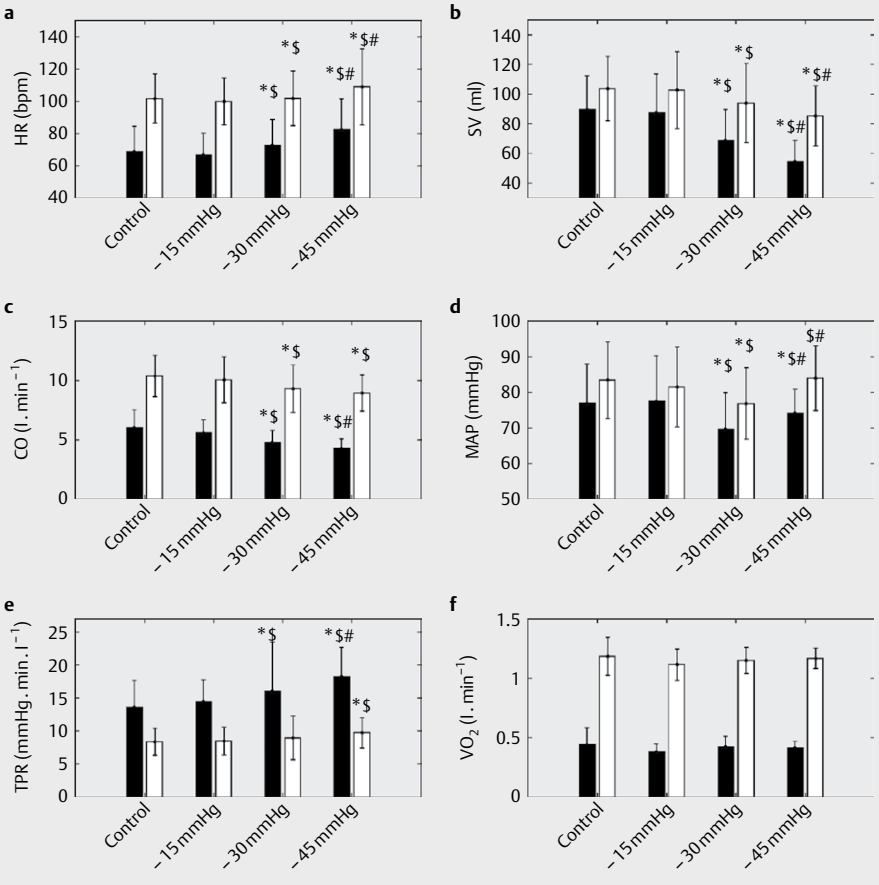
At rest, LBNP progressively induced an increase in HR and a decrease in SV. At −30 and −45 mmHg LBNP, with respect to Control, HR was higher ($p < 0.05$), whereas SV was lower ($p < 0.05$). Consequently, the resting CO was lower at −30 and −45 mmHg LBNP than in Control ($p < 0.05$). TPR increased with LBNP exposure at rest, resulting in higher values at −30 mmHg LBNP and −45 mmHg LBNP than in Control (▶ **Fig. 3e**, $p < 0.05$).

Similar trends were observed for the exercise steady state. MAP was slightly but significantly higher during exercise than at rest in all conditions (▶ **Fig. 3d**). TPR was lower during exercise than at rest. It was higher at −45 mmHg LBNP than in Control ($p < 0.05$). In all conditions, $Ca_{O_2}\text{-}\cdot C\bar{v}_{O_2}$ was higher during exercise than at rest.

▶ **Table 3** Steady state values of cardiopulmonary parameters at rest and during exercise in supine posture (Control).

		Rest	Exercise
HR	(min^{-1})	69 ± 16	102 ± 15 *
SV	(ml)	90 ± 23	104 ± 22
CO	($l\,min^{-1}$)	6.04 ± 1.49	10.40 ± 1.74 *
MAP	(mmHg)	77 ± 11	83 ± 11
TPR	(mmHg $min\,l^{-1}$)	13.7 ± 4.0	8.4 ± 2.1 *
$\dot{V}O_2$	($l\,min^{-1}$)	0.44 ± 0.15	1.19 ± 0.16 *
$CaCO_2\text{-}\cdot C\bar{v}O_2$	(ml)	73 ± 15	116 ± 20 *
$PaCO_2$	(mmHg)	37.5 ± 3.8	39.0 ± 3.9
pH		7.44 ± 0.02	7.43 ± 0.02
$[La]_b$	(mM)	1.14 ± 0.30	1.13 ± 0.58

HR, heart rate; SV, stroke volume; CO, cardiac output; MAP, mean arterial pressure; TPR, total peripheral resistance; $\dot{V}O_2$, oxygen uptake; $CaCO_2\text{-}\cdot C\bar{v}O_2$, arterial-venous oxygen difference; $PaCO_2$, arterialized CO_2 partial pressure; $[La]_b$, blood lactate concentration. * $p < 0.01$ compared to rest condition.



▶ **Fig 3** Rest (black bars) and exercise (white bars) steady state group values for heart rate (HR, panel **a**), stroke volume (SV, panel **b**), cardiac output (CO, panel **c**), mean arterial pressure (MAP, panel **d**), total peripheral resistance (TPR, panel **e**) and oxygen uptake ($\dot{V}O_2$, panel **f**) for 0 (Control), −15, −30 and −45 mmHg LBNP. Error bars represent SD. *, significantly different from Control; $, significantly different from −15 mmHg LBNP; #, significantly different from −30 mmHg LBNP.

No differences were found among conditions for arterialized $[La]_b$ and pH. The overall mean values, respectively at rest and exercise, were 1.01 ± 0.43 mM and 0.96 ± 0.43 mM for $[La]_b$, 7.44 ± 0.03 and 7.43 ± 0.02 for pH. Also $PaCO_2$ showed no differences among conditions, either at rest or during exercise. The overall mean was 37.5 ± 3.6 mmHg and 39.3 ± 3.6 mmHg, respectively at rest and exercise, being significantly higher in the latter case.

Discussion

The results of this study were coherent with the tested hypothesis, as long as there was: 1) a progressive increase in A_1 for SV as a function of the LBNP level, 2) a clear trend toward a reduction of A_1 of HR with LBNP, 3) invariant time constants. Thus, the A_1 of CO did not change with increasing levels of LBNP, because of the opposite, compensatory changes of the SV and the HR A_1.

At − 15 mmHg LBNP, in which vagal control of HR is still predominant [26], the A_1 of HR was the same as in Control. Instead, the reduction in the A_1 of HR became evident at − 45 mmHg LBNP. This evidence is not such as to allow refutation of the vagal withdrawal conjecture, stating that the φ1 of HR results from the sudden withdrawal of vagal tone at exercise onset [11, 13]. This conjecture stemmed from experiments in which the HR kinetics at exercise onset was determined under full vagal blockade with atropine [13], and was reinforced by further work with different experimental approaches [11, 12, 40, 41]. In fact the function describing the time course of HR during φ1 may be more complex than an exponential. Fagraeus and Linnarsson [13] reported an initial peak, which disappeared under pharmacological vagal blockade, followed by a transient drop and thereafter by a subsequent increase to steady state, corresponding to φ2. This pattern was observed also by a few further studies [18, 42]; however, it did not appear in the present study, as in several previous studies [11, 12, 43–45], just to cite a few.

Nevertheless, the progressive increase of A_1 of SV as a function of the LBNP level was very clear and significant. Under LBNP, a greater amount of blood is accumulated in the lower limb muscles than in Control [46–48]. The stronger the LBNP exposure, the larger would be the amount of blood displaced at the beginning of exercise toward the heart, characterized by low central venous pressure [49]. This would determine a stronger mechanical stimulus on the heart walls, which may result in increased SV, in agreement with the heart filling conjecture. The origin of this mechanical phenomenon may be multifactorial: the Frank-Starling mechanism, the ventricular interdependence and the pulmonary circulation could be considered as main actors in this process [15, 50–52]. The Frank-Starling mechanism implies that the force developed during a contraction of the left ventricle increases when the myocardial fibers are more stretched before that contraction than before the preceding one. However, the venous blood enters the right side of the heart, which interacts with the left side of the heart. Right side venous return enters the left ventricle only after a few beats, and only then the left ventricle wall is directly stretched. Sundblad et al. [52], who studied subjects during rest and leg exercise, as they were suddenly tilted from upright to supine, found an initial drop in SV, which they attributed to ventricular interdependence [15, 50–52]. Moreover, pulmonary circulation, a remarkable blood reservoir, may act as a buffer [53], further attenuating the effect of venous return on left ventricular contraction. On the other hand, Elstad et al. [43], who performed exercise with small proximal muscle masses, stressed the role of muscle vasodilation and the ensuing rapid TPR fall in determining the φ1 increase in SV. They suggested that the fall of MAP would be the primary factor involved in SV dynamics instead of the effects of muscle pump action on ventricle expansion and muscle fiber stretching.

We note, however, that TPR during exercise steady state was higher, the higher the LBNP level (▶ **Fig. 3**). This trend should reduce the size of the SV response in φ1, if it was mostly due to TPR changes. Moreover, the fall of MAP did not precede the increase in SV, as the nadir of MAP was attained when the SV changes in φ1 were already complete. Thus, we postulate that the increase in A_1 of SV, observed under LBNP, represents the most direct non-invasive evidence obtained so far in healthy humans that the φ1 increase in SV may have a mechanical origin, in agreement with the heart filling conjecture.

Recently, the occurrence of a tonic inhibitory muscarinic influence on left ventricular contractility was demonstrated [54]. This finding carries along the alternative hypothesis that, the stronger the degree of vagal withdrawal, the larger the SV response at exercise onset. This hypothesis is in contrast with the present data. In fact, since resting vagal activity is reduced under LBNP [26], implying a reduced reserve for vagal withdrawal, according to Machhada's hypothesis [54], one should find a reduction of A_1 of SV during exercise transients under LBNP: however, this was not the case.

The SV response to exercise, contrary to that of HR, may be nonlinear [6], and thus the SV response to a sudden increase in venous return may be more complex than implied by a single-exponential function. This being so, application of an exponential model to the analysis of SV in φ1 may be simplistic. In fact, Lador et al. [12] refrained from fitting parameters through SV data and evaluated the contribution of SV to the A_1 of CO, using the following equation:

$$CO_r + \Delta CO = (SV_r + \Delta SV)\,(HR_r + \Delta HR) \qquad (3)$$

where CO_r, SV_r, and HR_r are the resting values, and ΔCO, ΔSV and ΔHR stand for the respective increments during φ1, which should correspond to A_1. Thus, substitution of the A_1 of CO for ΔCO and of the A_1 of HR for ΔHR in equation 3 allowed an independent computation of ΔSV. ▶ **Table 4** shows that ΔSV did not differ from the corresponding A_1 of SV, as calculated by fitting of the double-exponential model, suggesting that the exponential fitting provides correct values for the SV changes during φ1.

The τ_1 of CO can be considered equivalent to that of $\dot{V}O_2$, given that the minimal time window in which $\dot{V}O_2$ can be determined is one breathing cycle. In fact, the τ_1 of $\dot{V}O_2$ was functionally instantaneous, indicating a practically immediate upward translation of $\dot{V}O_2$ that appeared since the first breath (▶ **Table 1**). Thus, the $\dot{V}O_2$ kinetics in φ1 may indeed be imposed by the φ1 kinetics of CO, in full agreement with the cardiodynamic hypothesis of lung $\dot{V}O_2$ kinetics [1]. The results that Drescher et al. [55] obtained using different tilt angles instead of LBNP are, however, in apparent contrast to the present data. These authors, who concluded that there are no postural effects on the HR and $\dot{V}O_2$ kinetics, analyzed their data

▶ **Table 4** Comparison between the amplitudes (A_1) of the phase I (φ1) and the increment (Δ) during φ1, as calculated using equation 1 or 2, respectively for stroke volume (SV) and oxygen uptake ($\dot{V}O_2$), with and without lower body negative pressure.

		Control	−15 mmHg	−30 mmHg	−45 mmHg
SV	A_1 (ml)	6.8 ± 3.5	9.9 ± 4.6	14.6 ± 7.3	19.8 ± 10.0 * #
	Δ (ml)	6.7 ± 12.8	10.3 ± 9.5	17.6 ± 8.3	20.0 ± 11.8 *
$\dot{V}O_2$	A_1 (l min^{-1})	0.22 ± 0.12	0.23 ± 0.08	0.26 ± 0.12	0.35 ± 0.15
	Δ (l min^{-1})	0.38 ± 0.18	0.33 ± 0.10	0.41 ± 0.17	0.33 ± 0.20

Values are means ± SD. No statistical differences were found between calculated and measured data (A_1). * $p<0.05$ compared to Control group; # $p<0.05$ compared to −15 mmHg.

with the cross-correlation transfer procedure they previously described [56]. Therefore, their conclusion does not apply to φ1, which is the object of the present study, but to what in the present model corresponds to the φ2 of the HR and $\dot{V}O_2$ kinetics, which covers a different domain of the exercise transient from the present one.

The steady state results at rest and exercise agree with previous data and represent a remarkable quality check of the present quantitative analysis during φ1. At rest, we found progressive reduction of SV and increase in HR with increasing levels of LBNP [57–59]. Since the effect of LBNP was more evident on SV than on HR, CO became lower, the higher the LBNP level, in agreement with previous findings [58–60]. These results, possibly via a baroreflex inhibition, might have entailed a reduced vagal activity and increased sympathetic activity under LBNP at rest [26, 61, 62]. Accordingly, TPR was higher under LBNP than in Control (▶ **Fig. 3**), both at rest and during exercise.

During exercise in LBNP, MAP followed similar patterns to those described by Mack et al. [63], although in the present study MAP was lower than in their study. In this study, MAP was lower at −30 mmHg than in Control, which was not the case when the negative pressure was set at −45 mmHg. Conflicting results appear in the literature. MAP was found to be unaffected by a mild LBNP level (between −10 and −20 mmHg [64]), whereas stronger LBNP levels (from −20 mmHg and down to −50 mmHg) induced either an important decrease in MAP and increase in HR [64], or no changes of brachial MAP measured invasively [59, 63].

Conclusions

We conclude that the φ1 kinetics of SV at exercise onset is compatible with the heart filling conjecture, whereas the vagal withdrawal conjecture for the φ1 kinetics of HR cannot be dismissed. Therefore, the φ1 kinetics of CO, characterized by the same τ_1 as that of SV and of HR, and by an A_1 predictable from those for SV and HR, may indeed result from two independent yet parallel mechanisms. The CO increase during φ1 engenders and then explains the φ1 increase in $\dot{V}O_2$, which is the consequence of the rapid cardiovascular phase. This implies that the $\dot{V}O_2$ and CO kinetics analysis at exercise onset should be performed considering these two different mechanisms: with the LBNP it is possible to act modifying predominantly the mechanical component of the rapid cardiovascular response, although at the highest LBNP level (−45 mmHg) the reduction of the vagal tone becomes substantial. This is the strongest demonstration provided so far in support of the concept of a dual control of CO kinetics in the early phase of exercise in humans.

These data support the hypothesis that at the early onset of exercise two parallel mechanisms act in preventing the fall of CO. The heart filling conjecture would become one of the most relevant mechanisms in conditions characterized by a lower vagal activity, such as ageing, hypoxia [12, 16, 17], and prolonged bed rest [48]. In these cases, the impairment of the neural component limits the correction of the fall in arterial pressure at exercise start, thus increasing the subsequent risk of syncope.

Funding

This study was supported by Swiss National Science Foundation Grants 32003B_127620 and 3200B0-114033 to Guido Ferretti.

Conflict of Interest

The authors declare no conflict of interest.

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