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Research article

The role of active muscle mass on exercise-induced cardiovascular drift

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Abstract
The purpose of this study was to examine the role of active muscle mass on cardiovascular drift (CV_{drift}) during prolonged exercise. Twelve subjects with peak oxygen uptake (VO_{2peak}) of 3.52±0.52 L·min^{-1} (mean ± SD) cycled for 55 min with 80 revolutions per minute with either two legs (2-legged) or one leg (1-legged). Oxygen uptake was at 60% of VO_{2peak} throughout the 2-legged trial and at half of this value in 1-legged condition. Cardiac output (CO-CO_2 rebreathing), heart rate (HR) and quadriceps integrated electromyographic activity (iEMG) were higher ($p < 0.01$) during 2-legged than 1-legged exercise. Changes in stroke volume from 20 to 50 min of exercise were greater in 2-legged than in 1-legged (ΔSV: -20.8±0.8 vs. -13.3 ± 1.3 ml·beat^{-1}, $p < 0.05$). Similarly, changes in heart rate (ΔHR) were +18.5 ± 0.8 and +10.7 ± 1.0 beats·min^{-1}, in 2-legged and 1-legged, respectively ($p < 0.01$). Calculated blood volume changes declined significantly in 2-legged exercise (ΔBV: -4.25 ± 0.43%, $p < 0.05$). Sympathetic activation as indicated by the ratio of low and high frequency in spectral analysis of HR (LF·HF^{-1} ratio) was higher in 2-legged than in 1-legged trial ($p < 0.05$). At the end of exercise, CO had a tendency to decrease from 20th min in 2-legged (changes in CO = -0.92 ± 0.3 L·min^{-1}, $p = 0.07$), whereas it was maintained in 1-legged cycling (ΔCO = -0.15 ± 0.2 L·min^{-1}, $p = 0.86$). Multiple regression analysis showed that HR rise and blood volume decline were predictors of SV drop whereas heart rate increase was explained by rectal temperature and magnitude of muscle mass activation, as indicated by iEMG ($p < 0.05$) in 2-legged cycling. In conclusion, apart from the well-known factors of thermal status and blood volume decline, it seems that muscle mass involved plays also a role on the development of CV_{drift}.

Key words: Prolonged cycling, cardiovascular regulation.

Introduction

During prolonged steady state exercise, there is a progressive rise in heart rate (HR) and a decline in stroke volume (SV) followed often by a drop in cardiac output (CO) and mean arterial pressure (MAP). This phenomenon is called cardiovascular drift (CV_{drift}) and may impair performance (Ganio et al., 2006). CV_{drift} is known to be affected by hyperthermia (Gonzalez-Alonso et al., 1998), dehydration (Montain and Coyle, 1992) and increase in heart rate (Fritzsche et al., 1999). Moreover, blood pooling in the periphery (Rowell et al., 1969) is suggested as contributor to CV_{drift}. Interactions of these factors may also exaggerate CV_{drift} (Gonzalez-Alonso et al., 2000). Despite the contribution of the above-mentioned factors in the appearance of CV_{drift}, its cause is not fully understood (Coyle and Gonzalez-Alonso, 2001). CV_{drift} is more pronounced in cycling than in running despite the greater thermal load, dehydration and increased heart rate of the subjects in the latter type of exercise (Nassis and Geladas, 2002) suggesting that the active muscle mass plays a role. It is speculated that the magnitude of muscle mass involved in the exercise may be an important factor of CV_{drift} exaggeration and, therefore of impaired performance during prolonged muscular effort. Greater muscle mass participation results in higher sympathetic activation, which is stimulated by muscular reflex and central command as indicated by electrical action of the muscle and the expressed rate of perceived exertion (Franke et al. 2000; Nobrega et al., 1994; Pawelczyk et al., 1997). On one hand, oxygen uptake (VO_2) during 1-legged exercise is more than half of that measured during 2-legged cycling, inducing presumably marked differences in muscle energy turnover and respective metabolic responses (Lewis et al., 1985). On the other, Klausen et al. (1982) indicated that during cycling for 8-10 min with one and two legs at the same relative intensity (70% of VO_{2peak} specific to 1-legged and 2-legged condition), CO and HR responses were not statistically different. Consequently, haemodynamic response is not affected by muscle mass as long as exercise intensity is proportional to maximum capacity per leg. In order to distinguish the effect of muscle mass from that of exercise intensity, a paradigm of exercising with 1- and 2 legs at the same proportional absolute oxygen uptake should be adopted. Whereas paradigms asking the subjects to work at the same proportional absolute work rate have been used (oxygen consumption and relative intensity being different; Jensen-Urstad et al., 1994), there is lack of data concerning HR and CO during prolonged exercise performed at the same VO_2 per leg.

This study explored the role of active muscle mass on CV_{drift}. It was hypothesized that during prolonged one- and two-legged cycling at the same absolute VO_2 per leg, the CV_{drift} will be exaggerated during two-legged exercise due to a greater central command. It was further hypothesized that aggravated CV_{drift} during 2- compared to 1-legged exercise would be due to higher heart rate developed in the former condition.

Methods

Subjects

Twelve subjects with (mean±SD) age 23.4 ± 3.8 years, body mass 77.8 ± 7.3 kg, and VO_{2peak} of 3.52 ± 0.52 L·min^{-1} volunteered to participate in this study. All subjects gave their written consent to participate in the ex-

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periments after being informed with details about the procedure and possible risks involved.

Pre-experimental sessions

Prior to the main trials, each subject visited the laboratory four to five times for familiarization with instrumentation, methodology, and anthropometric evaluation. In addition, each subject performed two-legged submaximal (4-stages with 4 min duration), or maximal exercise on an electrically braked cycle ergometer (Lode RH, the Netherlands) to determine the oxygen cost-work rate relation and the VO_{2peak}. VO_{2peak} was determined as the highest VO_2 average of the 30 s, provided that the following criteria were fulfilled: 1) a plateau in VO_2 despite an increase in work rate, 2) respiratory exchange ratio higher than 1.1, and 3) blood lactate concentration higher than 9 mmol·L^{-1}. Lactate was measured (Accutrend, USA) 3 minutes after the completion of exercise

Procedure

Three days prior to the main trial, the subjects were asked to record their diet and physical activity and replicate these habits before each experiment. In addition, the subjects had to abstain from physical activity, caffeine and alcohol consumption during the day before the experiments. The experiments were conducted at the same time of day, at least 3 hours after a light meal. After the subjects emptied their bladder, their body mass was measured (Bilance Salus, Italy) and electromyography (EMG) and electrocardiography (ECG) electrodes were fixed to the knee extensors (vastus lateralis and vastus medialis) and to the chest, respectively. Also, a thermistor was placed in the rectum, at a depth of 13-15 cm from the sphincter. Following this preparation, the subjects warmed up on the cycle ergometer for 8 min at 50% of their VO_{2peak}. After 5 min stretch, maximal voluntary isometric knee extension (MVC) was determined for knee extensors using a modified strength-training device connected with a calibrated load cell. Three 5-sec maximal trials with strong verbal encouragement were conducted with one-minute rest, in order to obtain the maximum EMG signal. A fourth trial was conducted if there was a difference greater than 5% between trials and then the two trials with the higher force were averaged. Afterwards, subjects relaxed for about 20 min on the cycle ergometer while instrumented. Duplicate 10 μl and 50-60 μl capillary blood samples were collected from the right index finger for haemoglobin (Hb) and haematocrit (Hct) determination, respectively. In the first condition, subjects cycled at 80 rpm for 50-55 min with two legs at 60% of their 2-legged VO_{2peak}. The second condition was conducted at least three days later and the external workload was adjusted in order to attain the half of the VO_2 observed in the first condition. The same oxygen uptake per leg was adopted in order to avoid higher metabolic response and energy turnover demands in 1-legged condition in case that half work rate (Watt) per leg was selected (Jensen-Urstad et al., 1994). The absolute exercise intensity was 126±1 Watts in 2-legged exercise, whereas in the 1-legged condition was calculated according to Jensen-Urstad et al., (1994) to be ~9% less than the half of the absolute intensity in 2-legged (work rate: 126/2-9% = 57 Watt). During the 1-legged condition, the non-exercising foot was rested comfortably between pedals on the bike frame, and the return of the pedal was achieved by elastic springs attached to hand-bar. Subjects were familiarized with the experimental setup in 1-legged exercise at least one day before the experiment for 20 min. To secure the development of CV_{drift} subjects cycled without fan or fluids. In all experiments, the environmental temperature was 23.5 ± 0.4 °C and the relative humidity 56.2 ± 4.1%.

CO was determined from 15-22, 30-37 and 47-54 min using the exponential CO_2 rebreathing method (Defares, 1958) with triplicate measurements separated by 2 min to ensure physiological steady state. In case those measurements differed more than 5%, a fourth maneuver was performed and all trials were averaged. VO_2 was measured breath by breath and averaged every 10 sec at 1-8 min of exercise and, thereafter, 2-3 min before each CO determination. Muscle oxygenation (oxygen saturation, StO_2) and blood volume (total hemoglobin, HbT) were measured by near-infrared spectroscopy. One minute EMG activity was stored at the 15th, 25th and 40th min. Double measurements of blood pressure were taken at rest, at the ~25th min (before CO measurement) and before the end of exercise with an automated system (Criticon Dinamap, Finland) in a standardized position with the arm supported by the researcher at the level of the heart. In case of a difference between measurements greater than 5mmHg, a third measurement was conducted. HR (Polar S-810, Finland) and body temperatures were recorded every 5 min. Moreover, HR was recorded beat by beat in a 5 min period at rest and at 8-13 and 37-42 min of exercise. These HR data were used for HR variability (HRV) analysis. At the same periods, respiratory frequency (Rf) was measured at 500 Hz with a fast response thermocouple (TSD 202A, Biopac systems, USA) attached to the respiration valve. Forearm skin blood flow ($F_{sk}BF$) was recorded with Laser Doppler Flowmetry at rest and for 1 min at ~15 and 44 min of exercise. The general and local (legs) feeling of fatigue was expressed every 5 min using the Borg scale (RPE).

Analytical methods and equipment

A metabolic cart was used for the measurement of VO_2 (MedGraphics, CPX-D, USA), which was calibrated before exercise with two different gas mixtures. CO was determined with the CO_2 rebreathing method (Defares, 1958) as modified by DaSilva et al., (1985) and Nassis and Geladas, (2002). Subjects breathed from a bag containing 1.5-2 times the tidal volume at 50 breaths per minute, set by a metronome. According to CO_2 exponential method, CO was calculated using the indirect Fick equation. The three measurements of CO at a specific time period were averaged. The coefficient of variation in different exercise intensities varied from 2.4 to 3.6%, which ensures the reliable measurement of CO, given that the respective values in a direct method are 5-10% (Warburton et al., 1999).

Changes of StO_2 and HbT were recorded using near infrared spectroscopy (NIRS) (In Spectra^{325}, Hutchinson Technology Inc, USA). The operational principles of NIRS device are given elsewhere (Kounalakis et al. 2008). The 25 mm NIRS probe was attached and se-

cured with an adhesive patch to the shaved and cleaned skin in the medial aspect of the rectus femoris muscle. Before each testing the NIRS device was calibrated with a single light-scattering standard and validated against standard references equivalent to 38% and 90% hemoglobin saturation.

EMG activity, indicating relative muscle activation level, and MVC of knee extensors were measured in knee angle of 60° (0° = fully extended). Velcro straps were used to stabilize the subject and the mechanical response was recorded by a force cell (LC-500F, Kyowa, Japan) and by a dynamometer (SS-25, Biopac, USA). EMG was recorded using circular electrodes (Kendall-Arbo, Germany) (bandwidth of 20-500 Hz sensitivity of 0.08 mV) placed over the vastus lateralis (VL) and medialis (VM) belly with an inter-electrode distance of 20 mm. Before electrode placement, the skin overlying the muscles was carefully shaved and cleaned with an alcohol swab. A 50-Hz line filter was applied to the EMG data to prevent interference from electrical sources. EMG and force signals were digitized online (TEL 100, BIOPAC System, USA), sampled at 1 kHz and stored for further analysis. EMG signals were high pass filtered with a cut off frequency of 0.2 Hz and then smoothed with a low pass filter with a cut off frequency of 400 Hz (Acknowledge 7.3.3 Software, Biopac USA). The same EMG analysis for 15 consecutive cycles during cycling was conducted. All EMG data were normalized by dividing the value at each time point during cycling by the averaged EMG value obtained during the MVC and expressed as a percentage.

Five min fast Fourier transformation was used for HRV analysis, which is considered to assess autonomic influences of the heart (Carter et al., 2003). An ECG100 unit (Biopac, USA) amplified the signal from electrodes and digitized it online at 1 kHz. Frequencies of 0.04-0.15 Hz (LF-ms^2) and 0.15-0.40 Hz (HF-ms^2) were normalized according to total power spectrum and very low frequencies (<0.04 Hz, VLF-ms^2): LFn=LF/(total power-VLF)x100 and HFn= HF/(total power-VLF)x100. The ratio of LF·HF^{-1} was used as an index of the degree of sympathetic activation (Carter et al., 2003). Spectral analysis of the respiratory signal was performed by the same procedure. These spectra were used to assess the main respiratory frequency and to locate the respiratory component of the power spectral analysis of respiratory rate-interval variability.

Rectal temperature (T_{re}) was measured with a thermistor (Yellow Springs, USA) connected to a telethermometer (Yellow Springs, Model 46, USA). F_{sk}BF was estimated from the microvascular blood cell velocity measured with a Laser Doppler probe (LDF 100A, BIOPAC System, USA) placed on the dorsal side of the right arm and secured to remain in the same position and in full contact with the skin for the experimental trial. Laser Doppler was appropriately calibrated before its use.

The laser Doppler probe was connected to a LDF100A flow module (BIOPAC, USA). All sensor signals collected were first elaborated by a UIM100A A/D interface (BIOPAC) which was connected to the MP100A data acquisition unit (BIOPAC) for storage and further analysis. Hb concentration was determined with the cyanmethaemoglobin method (Dr. Lange Mini-Cuvette, LKM 143, Germany). Hct values were obtained via a reader (Hawksley, UK) after a 4-min centrifugation at 11500 rpm. Coefficients of variation in repeated analyses were 1.8% and 1.7% for Hb and Hct, respectively.

Calculations

SV (ml·beat^{-1}) was the ratio of CO over HR. MAP was calculated from systolic (SP) and diastolic pressure (DP): MAP=DP+1/3·(SP-DP). Total peripheral resistance (TPR) was MAP·CO^{-1}, and arteriovenous oxygen difference (a-vO_2Diff) was VO_2·CO^{-1}. Changes in plasma and blood volume were calculated using Hb and Hct data (Dill and Costill, 1974).

Statistical analysis

A two-way analysis of variance with repeated measures on both factors was used (Statistica 5.0, USA). Analysis of Covariance with repeated measures was also employed for ΔSV with oxygen uptake as covariate, for HR with T_{re} as covariate and for HRV using the Rf as a covariate. A Tukey test was employed to assign specific differences in the analysis of variance. Data are presented as means ± SD. Backward stepwise multiple regression analysis was conducted using as dependent variable the changes in HR and, as independent variables, the T_{re}, EMG activity, LF·HF^{-1} ratio and BV and PV changes. The same procedure was conducted with changes in SV as dependent variable and ΔHR, ΔT_{re}, ΔBV, ΔSBF, LF·HF^{-1} ratio as well as ΔHbT as independent variables. Significance level was set at 0.05.

Results

The mean VO_2 was 1.04 ± 0.09 and 2.1 ± 0.12 L·min^{-1} for 1- and 2-legged condition, respectively, with no changes over time. The intensity in 2-legged cycling was at 58% of 2-legged VO_{2peak} and the respective intensity for 1-legged condition was calculated according to Klausen et al., (1982) to be ~42% of 1-legged VO_{2peak}.

CO was higher in 2-legged condition ($p < 0.01$) with a tendency to decline (0.9 L drop) at the end of exercise ($p = 0.07$, Figure 1a). In both conditions SV dropped after the 35th min in 2-legged and at the 50th min in 1-legged exercise (Figure 1b). At the 50th min of exercise ΔSV was -20.8±0.8 ml·beat^{-1} in the 2-legged and -13.3±1.3 ml·beat^{-1} in the 1-legged exercise. HR had the reverse course of SV with differences between conditions ($p < 0.05$) (Figure 1c). The HR drift was still greater in 2-legged exercise when the percentage of VO_2, was used as a covariate ($p = 0.04$).

MAP was 101 ± 1.3 and 100.4 ± 1 mmHg in 1-legged and 2-legged condition, respectively, without significant differences over time. TPR was higher ($p < 0.05$) in 1-legged exercise at the 20th min (8.42 ± 0.38 vs. 5.92 ± 0.27 mmHg·L·min^{-1}), as well as at the 50th min (9.2 ± 0.41 vs. 6.26 ± 0.31 mmHg·L·min^{-1}) of cycling. In contrast, a-vO_2Diff was higher in 2-legged condition (8.7, 8.6 and 9.2 and 11.2, 11.8 and 12.4 ml of O_2 per 100 ml of blood for 1- and 2-legged conditions at 20, 35 and 50th min, respectively, $p < 0.01$).

The differences between conditions in StO_2 were 7-10% ($p < 0.05$) from the beginning of exercise, while the

respective differences in HbT did not reach statistical significance. Both variables kept increasing from the 25th min (Figure 2).

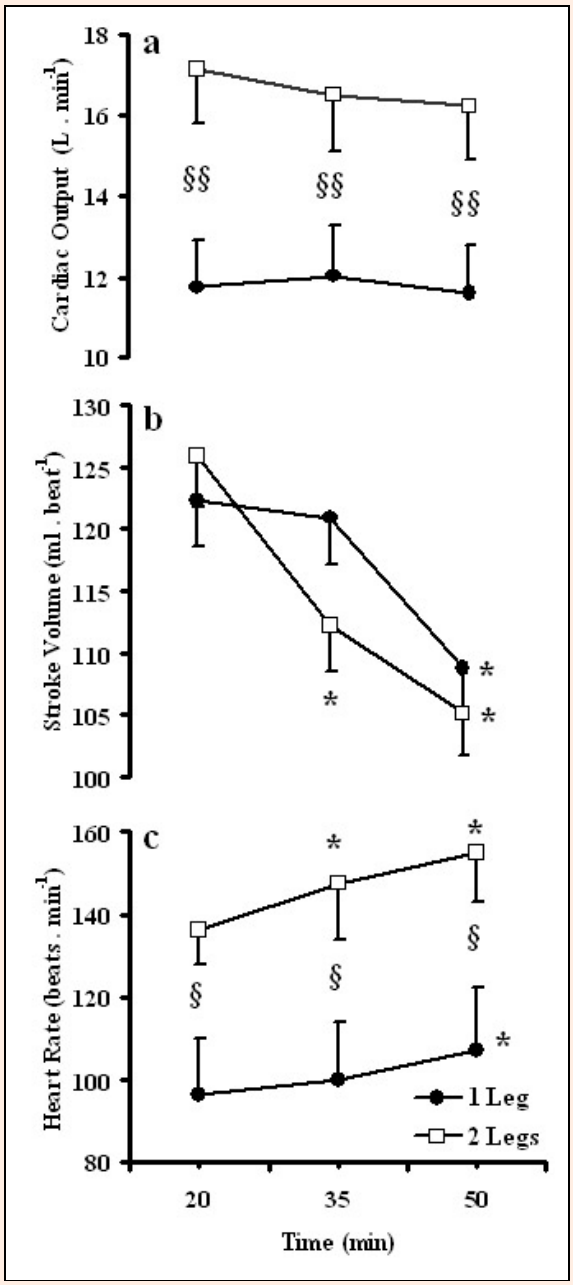

Figure 1. Cardiac output, stroke volume and heart rate during 55 min of cycling with 2 and 1 leg at similar oxygen uptake per leg (n = 12).
§ and §§ denote differences between conditions; $p < 0.05$ and $p < 0.01$, respectively. * denotes differences from the 20th min, $p < 0.05$

Integrated EMG activity was higher for VM and VL in the 2-legged condition (Figure 3) due to higher external workload applied. The $LF \cdot HF^{-1}$ ratio was increased from rest in both conditions with higher values observed in 2-legged cycling (2.98 ± 0.45, 5.23 ± 0.54 and 5.84 ± 0.58 for 1-legged vs. 3.40 ± 0.39, 8.04 ± 0.49 and 8.22 ± 0.86 for 2-legged condition at rest, at 9-14th and at 35-40th min, respectively).

Percent of plasma and blood volume decline from the pre-exercise values were significant in the 2-legged condition, while differences between conditions were observed only in the first variable (Table 1).

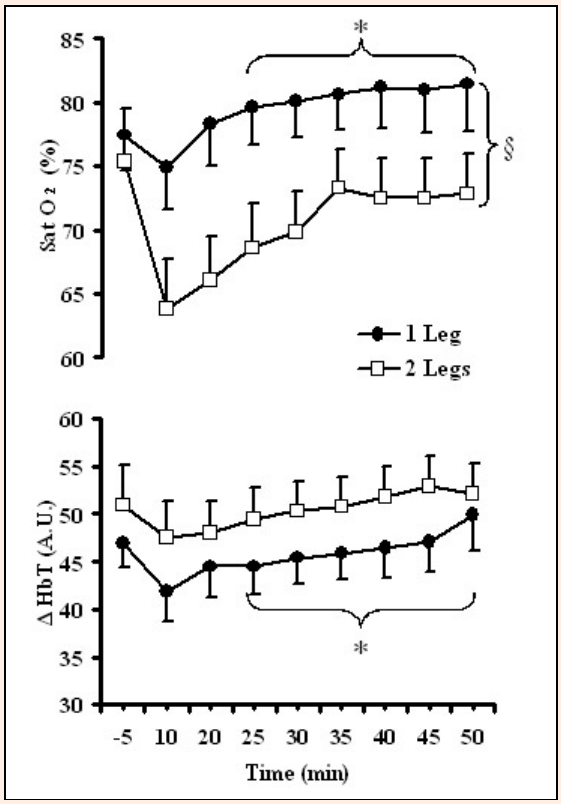

Figure 2. Muscle oxygen saturation (StO_2) and changes in blood volume (ΔHbT), during 55 min of cycling with 2 and 1 leg at similar oxygen uptake per leg (n = 12).
§ denotes differences between conditions; $p < 0.05$. * denotes differences from the 10th min, $p < 0.05$

At the end of exercise rectal temperature was higher compared to rest in both conditions ($p < 0.05$). At the 35th min of exercise and afterwards, T_{re} was higher in the 2-legged condition (Table 1). $F_{sk}BF$ increased throughout exercise compared to rest in both trials, but no difference was detected between conditions. The general and local RPE were different between conditions after the 30th min of exercise. The mean values of local and general RPE for one and two leg exercise were 12 ± 0.5 vs. 13.7 ± 0.3 and 10.5 ± 0.4 vs. 11.8 ± 0.4, respectively.

In the 2-legged condition, multiple regression analysis revealed that ΔSV was influenced ($r^2 = 0.99$, $p < 0.001$) by ΔHR ($p < 0.01$), ΔBV ($p = 0.04$) and ΔHbT ($p = 0.02$), while ΔHR was explained ($r^2 = 0.88$, $p < 0.01$) by ΔT_{re} ($p < 0.01$) and ΔEMG activity ($p < 0.01$). In 1-legged condition, ΔSV was explained ($r^2 = 0.99$, $p < 0.001$) by ΔHR ($p < 0.01$), whereas none of the examined factors explained ΔHR.

Discussion

The main finding of this study was that cycling with large muscle mass exaggerates CV_{drift} as indicated by the greater rise in HR throughout the protocol, and the larger drop in SV at the end of two legs compared with one leg

exercise. The greater rise in HR is accompanied by higher sympathetic response and is mainly related to EMG activity and RPE scale. These factors indicate a greater central activation in the 2-legged exercise, under the present experimental conditions.

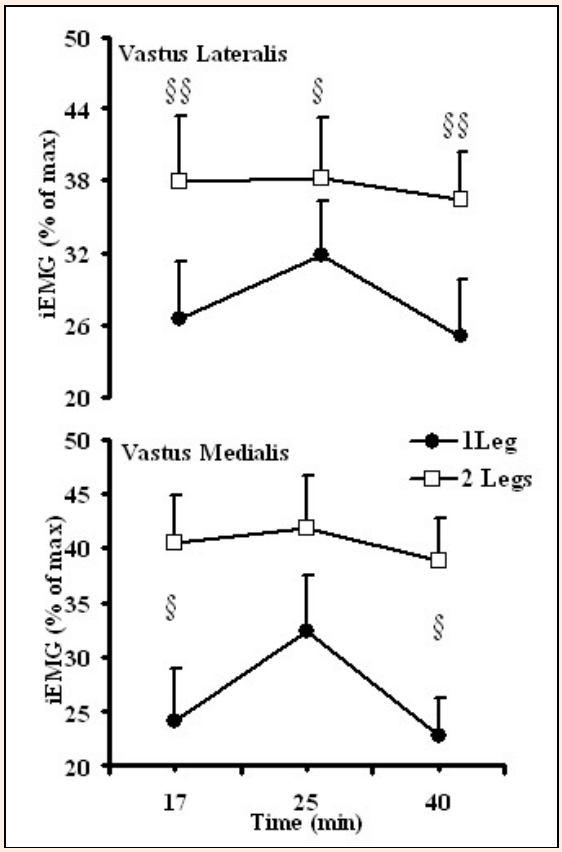

Figure 3. Integrated EMG (iEMG) in vastus lateralis and vastus medialis at the 17th, 25th and 40th min of exercise with 2 and 1 leg at similar oxygen uptake per leg (n = 12).
§ and §§ denote differences between conditions; $p < 0.05$ and $p < 0.01$, respectively. * denotes differences from the 17th min, $p < 0.05$

Central command activation could have affected sympathetic response, leading to heart rate increase and vasoconstriction in the muscle vascular beds (Pawelczyk et al., 1997; Schibye et al., 1981). The higher RPE observed in the 2-legged condition after the 30th min supports the greater central command hypothesis (Norton et al., 1999). Moreover, EMG activity was higher in the 2-legged condition (Figure 2), which means higher motor unit recruitment motivated by greater central command activation (Franke et al., 2000; Nobrega et al., 1994; Schibye et al., 1981). The association of muscle activation as recorded by the EMG activity and HR was confirmed by stepwise backward multiple regression analysis (n = 48), using the changes in HR as dependent variable, and T_{re}, iEMG, BV changes and RPE as independent variables. Regression analysis revealed that, besides T_{re}, EMG activity also explained almost the 50% of the variability in 2-legged HR (beta weight = 0.49 for EMG, $R^2 = 0.88$, $p < 0.01$).

Greater sympathetic drive is also indicated by the higher $LF \cdot HF^{-1}$ ratio in 2-legged exercise. This increase in sympathetic tone observed during the 2-legged conditions was likely necessary to maintain perfusion pressure and avoid hypotension (Calbet et al., 2004). Indeed, as in the present study, no differences in MAP have been observed between 1- and 2-legged cycling (Savard et al., 1989) despite the lower TPR and higher plasma catecholamines concentration in exercise with larger muscle mass (Jensen-Urstad et al., 1994). For instance, Savard et al. (1989) noted that during dynamic exercise, noradrenaline spillover (an indirect measure of sympathetic tone) is proportional to the fraction of the total muscle mass recruited without changes in MAP and this is not associated with any changes in limb blood flow. The higher VO_2 in 2-legged compared with 1-legged cycling is associated with larger reductions in StO_2 levels in the working muscles in the first condition (Figure 2a). The increase in a-vO_2Diff observed in the 2-legged condition implies adequate oxygen supply to the muscle since there were no significant differences in muscle blood flow between conditions, as indicated by similar HbT (Figure 2b). These observations are in agreement with studies reporting higher desaturation (Jensen-Urstad et al., 1994), a-vO_2 Diff (Jensen-Urstad et al., 1994; Lewis et al., 1983) and similar leg blood flow (Jensen-Urstad et al., 1994) with greater muscle mass participating in exercise. Norton et al. (1999) reported that during prolonged cycling, increased muscle afferent input to cardiovascular areas is also important for the development of CV_{drift} and, in that sense (Kjaer et al., 1999; Romer et al., 2007), the higher desaturation levels observed in the present study in the 2-legged condition, could also exaggerate the increase in heart rate and, therefore, SV decline. The significant relationship found between heart rate increase and ΔStO_2 ($r = 0.51$, $p < 0.05$) support this hypothesis.

Hyperthermia is another possible factor that could affect CV_{drift}. More specifically, the higher T_{re} observed

Table 1. Rectal temperature (Tre) blood volume (BV) and plasma volume (PV) changes from rest, and forearm skin blood flow ($F_{sk}BF$), at 20 (I), 35 (II), and 50 min (III) for T_{re}, at 35 min (II) for BV and PV, and at 15 (I) and 45 min (III) for $F_{sk}BF$. Values are means (±SD).

	Rest		I		II		III	
	1 Leg	2 Legs	1 Leg	2 Legs	1 Leg	2 Legs	1 Leg	2 Legs
T_{re} (°C)	37.0 (.2)	37.0 (.2)	37.3 (0.1)	37.4 (.2)	37.5 (.1)#	37.7 (.2) *#	37.6 (.1)#	37.9 (.2) *#
BV (%)	-	-	-	-	-2.07 (.68)	-4.22 (.41)#	-	-
PV (%)	-	-	-	-	-1.69 (.80)	-7.64 (.48)*#	-	-
$F_{sk}BF$ (AU)	5.5 (.8)	5.8 (.8)	29.7 (1.4)#	26.8 (1.1)#	-	-	26.8 (1.1)#	27.4 (1.1)#

* denotes differences between conditions, $p < 0.05$. # Differences from rest, $p < 0.05$. AU: Arbitrary units

in 2-legged than that in 1-legged cycling (by 0.3 °C) could have accelerated cardiac rhythm (Rubin, 1987) possibly by inducing a greater sympathetic response (Gonzalez-Alonso et al., 1999) as indicated by the $LF \cdot HF^{-1}$ ratio in the 2-legged condition. However, T_{re} cannot fully explain the different HR course between conditions because: a) HR was higher in 2-legged exercise at the same T_{re} and b) the statistical differences in HR between the 2 conditions remained when T_{re} was used as a covariate in analysis of variance of HR.

Similarly, the observed differences between experimental conditions in HR are not attributable to the higher exercise intensity (~15%) with 2 legs than 1 leg, since differences between conditions remained intact even when ANCOVA was used. These facts suggest that other factors than T_{re} and exercise intensity may play a role in the observed dissimilarities in heart rate increase in the two trials.

CV_{drift} was observed in both experimental conditions but it was more pronounced in 2-legged cycling (greater rise in ΔHR by 13 bpm). The resulting heart rate increase could reduce the cardiac filling time and explain the SV drop over time (higher drop in ΔSV by 7.5 $ml \cdot beat^{-1}$) (Franke et al., 2000). The high correlation ($n = 48$; 12 subjects x 4 time points) found between HR and SV changes in both conditions ($r = -0.80$ and -0.76 for 2- and 1-legged, $p < 0.01$) is in agreement with studies using prolonged cycling and running (Franke et al., 2000; Nassis and Geladas, 2002) and supports the role of heart rate increase in the development of SV drift under similar circumstances to the present study.

A greater BV reduction in 2-legged cycling could also induce increased heart rate via baroreceptor uploading (Norton et al., 1999) and thus explain the SV decline (Gonzalez-Alonso et al., 2000). As a support to the role of dehydration on SV reduction, backward stepwise regression analysis ($n = 48$, $R^2 = 0.97$, $p < 0.01$) showed that in 2-legged exercise, besides ΔHR (beta weight of 0.40, $p = 0.03$), and HbT (beta weight of 0.37, $p = 0.02$) ΔSV variability was explained also by ΔBV (beta weight of 0.2, $p = 0.04$), suggesting a role for HR drift.

The possible limitations of the present study include EMG activity and RPE measurements, which are not direct indexes of central activation. However, central command has been evaluated with indices similar to these used in the present study (Franke et al., 2000; Norton et al., 1999; Schibye et al., 1981). In addition, muscle blood volume changes were estimated with NIRS via HbT, which, however, is also considered as an index of muscle blood flow (Meyers et al., 2005).

Conclusion

In conclusion, during 55 min of cycling, CV_{drift} was exaggerated in 2-legged compared with 1-legged exercise at the same oxygen uptake per leg. It is implied that along with thermal status and blood volume decline, central command plays a role on cardiovascular regulation during steady state exercise performed with large muscle mass.

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Key points

- The magnitude of the participated muscle mass plays a critical role for the development of cardiovascular drift, when the oxygen consumption per leg is the same.
- Apart from thermal status and blood volume decline, central command plays a role on cardiovascular regulation during steady state exercise performed with large muscle mass.

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