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## Leg muscles activities during hyperventilation following a cycling exercise.

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29 **Abstract**

30 The goal of this study was to establish how increased ventilation modifies postural  
31 stability, as characterized by body sway and leg muscle activities. Twelve healthy  
32 subjects had to perform six 30-second postural tests: one pre-exercise test while  
33 breathing gently and then one test every minute for the five minutes immediately  
34 following a maximum-intensity, incremental cycling exercise test. Subjects were asked  
35 to maintain an upright stance on a force plate for 30 s, with their eyes open. Movement  
36 of the centre of pressure in the sagittal plane was monitored in the time and spectral  
37 domains. Myoelectric activities of the soleus and tibialis anterior muscles were recorded  
38 using surface electromyography. Ventilatory parameters were measured with a portable,  
39 telemetric device. Postural changes related to respiratory variations were quantified by  
40 coherence analysis. The results showed that hyperventilation induced by exercise was  
41 accompanied by a significant increase in postural parameters, indicating a reduction in  
42 postural stability following a change in ventilatory drive. Coherence analysis confirmed  
43 the ventilatory origin of the postural oscillations. The results suggest that ventilation  
44 may be an important factor in postural disturbance during physical activity. The  
45 observed increases in leg muscle activities were most likely related to musculo-articular  
46 stiffening.

## 47 **Introduction**

48 The ability to maintain balance plays a fundamental role in motor programming. In  
49 many sporting activities, this ability is considered as a key factor for supporting  
50 voluntary movement (3). In addition to gravity and other external forces, internal forces  
51 (related to voluntary movement and cardiorespiratory activities) may also disturb  
52 postural stability. In an upright stance at rest, it was well known that quiet breathing  
53 perturbs body balance in the sagittal and frontal planes and that the effects of  
54 compensatory motions of the trunk and lower limbs are more pronounced in the sagittal  
55 plane (2, 4, 10, 11). During high-intensity physical activity, however, the tremendously  
56 increased oxygen need translates into greater minute ventilation and deeper, faster rib  
57 cage movements. Hence, in principal, body mass distributions and maintenance of  
58 posture should be markedly perturbed during physical activity. Postural perturbation  
59 related to physical exercise has been demonstrated (7, 18). However, these studies are  
60 mainly focused on the effects of central and/or peripheral fatigue and have minimised  
61 internal perturbation linked to a deep and paced breathing. Thus, the aim of the present  
62 study was to investigate the extent to which an increase in ventilation might decrease  
63 postural stability. We therefore studied postural sway and leg muscle electromyograms  
64 (known to reflect postural strategies (12)) before and after a maximum-intensity,  
65 incremental cycling exercise test.

66

## 67 **Materials and Methods**

### 68 *Subjects*

69 Twelve healthy university students participated in this non-invasive study (mean age:  
70  $20.6 \pm 1.8$  yrs; mean weight:  $69.2 \pm 9$  kg; mean height:  $1.7 \pm 0.1$  m). All subjects had

71 been fully informed of the experiment's aims and procedures (in accordance with the  
72 Declaration of Helsinki) and gave their written consent. None of them presented any  
73 known diseases or injuries in their medical history that might have impaired balance or  
74 respiration. In order to evaluate the subjects' pulmonary capacity, routine spirometric  
75 tests by using a Fleisch pneumotachograph (Masterlab, Jaeger, France) were always  
76 performed by the same experimenter. The normality of parameters such as tidal volume  
77 ( $V_T$ ) or vital capacity (VC) was judged according to the reference values proposed by  
78 the European Respiratory Society (9).

#### 79 *Force plate data*

80 The ground reaction forces and their moments in the three planes (used to determine the  
81 coordinates of the centre of pressure, COP) were recorded using a rectangular (60 x 40  
82 cm), piezoelectric force plate (Kistler type 9281 B11, Kistler AG, Winterthur,  
83 Switzerland) linked to a charge amplifier (Kistler type 9851, Kistler AG, Winterthur,  
84 Switzerland). Since respiratory movements mainly concern the sagittal plane (11), we  
85 only considered the COP's anteroposterior displacement (COP<sub>x</sub>) recorded with a  
86 sensitivity of 50 mm/V.

#### 87 *Electromyography*

88 Electromyographic (EMG), bipolar recordings of soleus (SO) and tibialis anterior (TA)  
89 muscle activities were made on the dominant leg by using Ag/AgCl surface electrodes  
90 (Beckmann, 8 mm in diameter). Before placement of the latter, the skin at the electrode  
91 sites was prepared so as to achieve an inter-electrode impedance of less than 5 k $\Omega$ . Pairs  
92 of electrodes (centre-to-centre spacing: 20 mm) were respectively placed about 2 cm  
93 below the insertion of the gastrocnemius on the Achilles tendon for the SO and on the  
94 belly of the TA, parallel to the muscle fibres. A ground electrode was placed on the

95 medial surface of the tibia. EMG signals were differentially amplified (Gould™ 6600,  
96 Cleveland, USA) with a gain set to between 1000 and 5000 and filtered using a  
97 bandwidth of 10-1000 Hz. EMG and COP signals were simultaneously sampled at 1000  
98 Hz by using a 12-bit analogue-to-digital converter (National Instrument Daq Card™-  
99 AI-16E-4). The data were collected on a laptop computer interfaced with DataSet Pro  
100 software (SETRI, France) and then stored for off-line processing.

#### 101 *Ventilatory measurements*

102 Respiratory gas exchanges were monitored telemetrically using a portable gas analyser  
103 (Cosmed™ K<sub>2</sub>, Vacumetrics Inc., Ventura, CA, USA). The validity and reliability of  
104 measurements with the Cosmed K<sub>2</sub> have been demonstrated by a number of detailed  
105 studies (16). The Cosmed K<sub>2</sub> was carefully calibrated prior to each experiment. The  
106 subject wore the transmitting unit, battery and face mask (represented a total mass of  
107 850 g). The face mask sealed tightly over the subject's nose and mouth and was  
108 connected to a turbine flow meter. The latter did not add any significant, additional air  
109 flow resistance. Minute ventilation ( $\dot{V}_E$ ) was measured, along with its tidal volume  
110 ( $V_T$ ) and breathing frequency ( $f_R$ ) components. The values were averaged every 5  
111 seconds, stored and then transferred onto the laptop computer for further analysis. For  
112 each subject, the  $V_T$  to  $V_C$  ratio was used as an index of ventilatory change. The  
113 ventilatory flow signal was obtained via the Cosmed K<sub>2</sub> by measuring variations in  
114 timing breathing. When the subject expires through the face mask, the revolutions of the  
115 turbine produce a voltage signal. Conversely, when the subject inspires, the turbine  
116 stops and entails the cancellation of the voltage signal. The output signal is transmitted  
117 (simultaneously with COP<sub>x</sub> and EMG signals) to the 12-bit analogue-to-digital  
118 converter acquisition system.

119 *Experimental procedure*

120 Subjects had to perform a total of 6 postural tests, each corresponding to maintenance of  
121 a bipedal stance for 30 s (eyes open). A reference test (pre-effort postural test) was  
122 carried out in quiet conditions prior to a maximal incremental cycling exercise (ICE)  
123 test lasting about 10 minutes, i.e. enough to generate hyperventilation (HV). Five post-  
124 effort postural tests (PEPTs) were then carried out - immediately after the ICE (PEPT<sub>0</sub>)  
125 and then during the first 30 seconds of each of the next 4 minutes (PEPT<sub>1</sub> to PEPT<sub>4</sub>).

126 All postural tests were performed in a quiet room without sound and light  
127 interference. Subjects were asked to stand barefoot on the force plate, relax with their  
128 arms hanging alongside the trunk and focus on a target placed 2 m in front of the subject  
129 at eye level. The foot position was standardized by placing foot prints on the force plate,  
130 in agreement with the recommendations of the French Posturology Association (1). The  
131 foot prints (adjusted to the subject's foot length) were drawn so that the respective  
132 longitudinal axes formed a 30° angle and intersected the force plate's anteroposterior  
133 axis, with the heels 2 cm apart.

134 ICE on a cycle ergometer (Monark™ 824&, Sweden) was performed with  
135 cardiac monitoring until exhaustion, as judged by each subject's maximum oxygen  
136 uptake ( $\dot{V}O_2\text{max}$ ). This parameter was predicted by using the gender-adjusted  
137 formulae for cycle ergometer exercises reported by Wasserman et al. (20). Resting  
138 oxygen uptake ( $\dot{V}O_2\text{rest}$ ) was measured in a relaxed, upright stance using the Cosmed  
139 K<sub>2</sub>. After subtraction of  $\dot{V}O_2\text{rest}$  and by considering that each watt required 10.3  
140 ml/min of O<sub>2</sub> (19), the maximum workload (W<sub>max</sub>) was calculated as follows:  
141  $W_{\text{max}} = (\dot{V}O_2\text{max} - \dot{V}O_2\text{rest})/10.3$ . Subjects performed a 3-min warm-up (with a  
142 workload corresponding to 20% of W<sub>max</sub>) and then a 10-min period of incremental

143 exercise from 20 to 100% of Wmax, with an increased workload stage every minute.  
 144 For each stage, the added workload was  $(W_{max}-20\%W_{max})/10$ . The pedalling rate  
 145 was held at 60 revolutions per minute.

#### 146 *Signal processing*

147 The off-line processing of COPx, EMG and respiratory signals was performed using a  
 148 custom processing routine written with Matlab 6.5 (The Mathworks, Inc., Natick, MA,  
 149 USA). The respiratory and COPx signals were passed through a 4th-order low-pass  
 150 Butterworth filter with a cut-off frequency of 15 Hz. Over the 30 s of each postural test,  
 151 the mean amplitude (Ax) and the sway path (SPx) were calculated using the method  
 152 described by Hufschmidt et al. (13). The frequency content of the COPx displacements  
 153 was computed by Fourier analysis in order to determine the mean power frequency  
 154 (MPF). The power spectral densities of the respiratory and COPx signals were  
 155 calculated by periodogram method. Coherence analysis was used for measuring changes  
 156 in COPx related to respiratory variations. The magnitude squared coherence  $C_{xy}(f)$  of  
 157 the signals  $x$  and  $y$  was estimated at each frequency  $f$  as:

$$158 \quad C_{xy}(f) = \frac{|P_{xy}(f)|^2}{P_{xx}(f) \cdot P_{yy}(f)},$$

159 where  $P_{xx}(f)$  and  $P_{yy}(f)$  represent the power spectral densities of  $x$  and  $y$  respectively and  
 160  $P_{xy}(f)$  the cross power spectral density of  $x$  and  $y$ . The spectral densities can be obtained  
 161 by average Welch's periodogram. Coherence provides a normalised spectral measure  
 162 (ranging between 0 and 1) of the linear association between two signals as a function of  
 163 frequency, where 0 indicates that there is no association and 1 indicates a perfect  
 164 association. During the postural tests, muscle activation levels were quantified from the  
 165 raw EMG signals by computing the root mean square value (RMS) over 300 ms with a



166 sliding step of 20 ms. The RMS values were then averaged and expressed as  
167 percentages of each subject's baseline values acquired during the pre-effort postural test.  
168 Muscular fatigue was assessed through power spectral analysis of the EMG signals by  
169 calculating the MPF value.

#### 170 *Statistical analyses*

171 A simple repeated-measures analysis of variance was performed to assess the effects  
172 caused by ICE on ventilatory, electromyographic and postural sway parameters  
173 (Statview, SAS Institute Inc, Cary, NC, USA). Post hoc testing (the Student Newman-  
174 Keuls test) was used to identify local differences. Using the data from all subjects and  
175 all postural tests, Bravais-Pearson correlation coefficients ( $r$ ) were calculated in order to  
176 evaluate potentially linked parameters. For all statistical tests, differences were  
177 considered significant when  $p < 0.05$ .

178

#### 179 **Results**

180 The mean ( $\pm$  *SD*) power output produced by the subjects at the end of ICE was  $249.6 \pm$   
181  $49.9$  W. As shown in Table 1, measurements taken at PEPT<sub>0</sub> immediately following  
182 ICE prompted a significant increase in  $\dot{V}O_2$  (852%),  $\dot{V}_E$  (703%),  $V_T$  (260%),  $V_T/VC$   
183 (255%) and  $f_R$  (127%), ( $p < 0.01$ ). Significant increases in both Ax (54%,  $p < 0.05$ ) and  
184 SPx (168%,  $p < 0.01$ ) were observed. All ventilatory and postural parameters remained  
185 significantly higher ( $p < 0.05$ ) 4 minutes after ICE, with the exception of Ax, which  
186 returned to pre-effort values one minute after ICE. Linear regression analysis showed a  
187 number of significant correlations between ventilatory and postural sway parameters -  
188 particularly between  $\dot{V}_E$  and SPx and between  $f_R$  and SPx (Figure 1). The correlations  
189 between ventilatory parameters and Ax (ranging between 0.24 and 0.42) were always

190 lower than those found for ventilatory parameters and SPx (ranging between 0.51 and  
191 0.82).

192 In comparison with reference values, the spectral analysis of the COPx  
193 displacements revealed a significant increase in MPF (34%,  $p < 0.01$ ) which remained  
194 significantly higher 4 minutes after ICE ( $p < 0.01$ ). The results of coherence analysis  
195 demonstrated a high level of correlation ( $0.70 \pm 0.11$ ) between respiratory and COPx  
196 signals at PEPT<sub>0</sub> immediately after ICE over precise frequency range (0.5-0.7 Hz).  
197 Examples of spectral and coherence analysis are illustrated in Figure 2.

198 As shown in Figure 3, ICE also led to a significant increase in SO (190%,  $p <$   
199  $0.01$ ) and TA (87%,  $p < 0.01$ ) RMS values in all PEPTs. After normalizing RMS values  
200 with respect to Ax, comparisons with pre-effort values remained significantly high for  
201 both muscles ( $p < 0.05$ ). Electromyogram MPF mean ( $\pm SD$ ) values obtained prior and  
202 just after ICE were  $133.6 \pm 8.7$  Hz vs  $128.5 \pm 11.5$  Hz for SO ( $p > 0.05$ ) and  $97.2 \pm 10.2$   
203 Hz vs  $88.2 \pm 17$  Hz for TA ( $p > 0.05$ ).

204

## 205 **Discussion**

206 Hence, we observed that cycling exercise up to maximum oxygen consumption led to  
207 major adaptations in ventilation: notably, oxygen debt had a lasting impact on the  
208 postural control system. Indeed, the value of  $V_T/VC$  (revealing greater excursion of the  
209 rib cage motion) remained significantly higher than at rest throughout the 5-min  
210 recovery, providing a relevant model for post-exercise (metabolic) hyperventilation.

211 In agreement with other work (2, 11, 14, 15), increasing respiratory output  
212 reduced postural stability (as measured by SPx and Ax changes). Because of higher  
213 post-exercise ventilation, the body sway range quantified here was much higher than

214 those reported in previous studies (2, 15). Here, the postural parameters progressively  
215 returned to their initial values following the kinetic of the ventilatory recovery (as  
216 attested by correlations between  $\dot{V}_E$  or  $f_R$  and SPx), suggesting a ventilatory origin of  
217 postural instability.

218         Nevertheless, the reduction in postural stability following exercise could also  
219 correspond to leg muscle fatigue after cycling. Taking into account treadmill running  
220 and cycling exercise at 125 Watts, Nardone et al. (18) have only observed postural  
221 perturbation after running. They have attributed these differences to a lower oxygen  
222 uptake in cycling (65.2%  $\dot{V}O_{2max}$ ) compared to running (84.6%  $\dot{V}O_{2max}$ ) and to a  
223 lower peripheral fatigue of leg muscle in cycling. However, postural disturbance has  
224 been demonstrated after a prolonged cycling exercise (17) and the incremental cycling  
225 exercise used in the present study has been brought until ~250 Watts and 100% of the  
226 maximal oxygen uptake. Fatigue is known to alter the mechanisms involved in postural  
227 control (17). Studies of the effects of fatigue on the upright stance report an increase in  
228 postural sway (5, 6). For example, Caron (5) reported that isometric contraction of the  
229 plantar flexor until task failure increases the mean velocity of COP displacements by  
230 31%. The mean velocity values reported in our study at PEPT<sub>0</sub> showed an increase by  
231 170%. Thus, the effects of fatigue of the lower limbs induced by cycling exercise  
232 cannot alone explain such modifications. Furthermore, several studies related to  
233 voluntary hyperventilation (2, 15) - which should not induce leg muscle fatigue - have  
234 reported perturbed balance, suggesting a ventilatory origin. Hence, in the present study,  
235 muscle fatigue does not appear to be the main factor in postural disturbance. The high  
236 level of coherence found in the current study between respiratory and COPx signals  
237 over the low frequencies (0.5-0.7 Hz) clearly confirmed the ventilatory origin of the

238 COPx oscillations. Thus, our results suggest that ventilation may be an important factor  
239 in postural disturbance during physical activity.

240 Correlation analyses indicate that the sway path was better correlated with  $f_R$   
241 than with  $V_T$ , which is in good agreement with Jeong (14). This finding suggests that  $f_R$   
242 is the main factor disturbing balance in the upright stance. In comparison with SPx  
243 values, correlations between ventilation parameters and Ax were lower: at the start of  
244 the recovery period, Ax values did not significantly differ from their baseline values.  
245 One could suppose that the nervous system sets up earlier compensatory mechanisms to  
246 limit the amplitude (rather than the frequency) of the COPx oscillations. One possible  
247 mechanism is co-contraction of antagonistic leg muscles, since we observed a  
248 significant increase in the activation levels of both SO and TA. The increase in SO and  
249 TA electromyography activity is known to enhance musculo-articular stiffness of the  
250 ankle joint (21). Insofar as COP motion reflects the activity of the neuromuscular  
251 system, this could also explain the increase in spectral content of COPx motion towards  
252 high frequencies (as measured by MPF changes). However, the increase in leg muscle  
253 activities observed in our study after cycling could also reflect a recruitment of  
254 additional motor units and/or an increase in motor unit firing rates in response to muscle  
255 fatigue (5) but the spectral analysis of SO and TA electromyography activity -  
256 conducted before and immediately after ICE - revealed no significant change in the  
257 mean power frequency, an index used to detect muscle fatigue (8). That reinforces the  
258 hypothesis of a compensatory mechanism to decrease sway amplitude, rather than leg  
259 muscle fatigue enhancing postural instability.

260 In summary, we demonstrated that post-exercise model of metabolic  
261 hyperventilation leads to major postural disturbances, as notably reflected by increased

262 body oscillation in the sagittal plane. Coherence analysis proved to be a subtle method  
263 to show the close relationship between breathing and postural stability. It is likely that  
264 the strategy used by the nervous system to limit amplitude of body oscillation  
265 corresponds to recruitment of leg muscle motor units and a resultant increase in  
266 musculo-articular stiffness.

267

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324 **Figure captions**

325

326 Fig. 1. - Relationships between the sway path (SP<sub>x</sub>) of the COP<sub>x</sub> displacements and the  
327 minute ventilation ( $\dot{V}_E$  in panel A) or the breathing frequency ( $f_R$  in panel B) in twelve  
328 subjects. For each subject, the data were generated in a single pre-effort postural test  
329 and then five postural tests following incremental cycling exercise.

330

331 Fig. 2. - Example of spectral and coherence analysis applied on both respiratory and  
332 COP<sub>x</sub> signals at PEPT<sub>0</sub> for one subject. In panel A, the frequency distribution of the two  
333 signals (represented in grey for the ventilation and in black for the centre of pressure)  
334 revealed a synchronisation in spectral densities around 0.6 Hz. In panel B, illustration of  
335 coherence spectrum measurement between the respiratory and COP<sub>x</sub> signals obtained  
336 for the same subject. Its magnitude of 0.81 over the low frequencies (0.6 Hz) indicated a  
337 high level of correlation between these two signals.

338

339 Fig. 3. - Soleus (black bars) and tibialis anterior (grey bars) EMG activities measured in  
340 postural tests following incremental cycling exercise. EMGs were quantified by their  
341 RMSs and expressed as percentages of the baseline values obtained in the pre-effort  
342 postural test (dotted line). Means plus one standard deviation are represented. \* and \*\*  
343 denote significant differences between the pre-effort postural test and the post-effort  
344 postural tests at  $p < 0.05$  and  $p < 0.01$  respectively.



345 **Tables**

346 *Table 1 - Ventilatory ( $\dot{V}O_2$ ,  $\dot{V}_E$ ,  $V_T$ ,  $V_T/VC$ ,  $f_R$ ) and postural sway (SPx, Ax, MPF)*  
 347 *parameters measured in postural tests before (pre-effort test), just after (PEPT<sub>0</sub>) and*  
 348 *over the next 4 minutes following (PEPT<sub>1</sub> to PEPT<sub>4</sub>) an incremental cycling exercise*  
 349 *(mean  $\pm$  standard deviation). \* and \*\* denote significant differences between the pre-*  
 350 *effort postural test and the post-effort postural tests at  $p < 0.05$  and  $p < 0.01$*   
 351 *respectively.*

	Pre-effort test	PEPT <sub>0</sub>	PEPT <sub>1</sub>	PEPT <sub>4</sub>
$\dot{V}O_2$ (L <sub>STPD</sub> .min <sup>-1</sup> )	0.36 $\pm$ 0.05	3.43 $\pm$ 0.70**	2.35 $\pm$ 0.41**	0.66 $\pm$ 0.16**
$\dot{V}_E$ (L <sub>BTPS</sub> .min <sup>-1</sup> )	12.8 $\pm$ 1.4	102.8 $\pm$ 19.1**	71.0 $\pm$ 11.2**	28.4 $\pm$ 5.5**
$V_T$ (L <sub>BTPS</sub> )	0.75 $\pm$ 0.10	2.70 $\pm$ 0.65**	2.40 $\pm$ 0.61**	1.28 $\pm$ 0.30**
$V_T/VC$ (%)	14.5 $\pm$ 1.9	51.5 $\pm$ 9.0**	45.6 $\pm$ 8.4**	24.3 $\pm$ 4.4**
$f_R$ (Hz)	0.29 $\pm$ 0.05	0.66 $\pm$ 0.19**	0.52 $\pm$ 0.15**	0.38 $\pm$ 0.08**
SPx (mm)	221.3 $\pm$ 32.0	594.5 $\pm$ 101.2**	339.5 $\pm$ 120.4**	268.6 $\pm$ 45.2*
Ax (mm)	7.2 $\pm$ 2.4	11.1 $\pm$ 2.6*	6.7 $\pm$ 1.7	6.2 $\pm$ 2.0
MPF (Hz)	0.46 $\pm$ 0.06	0.62 $\pm$ 0.13**	0.56 $\pm$ 0.16**	0.59 $\pm$ 0.09**

352

## Figures

Figure 1

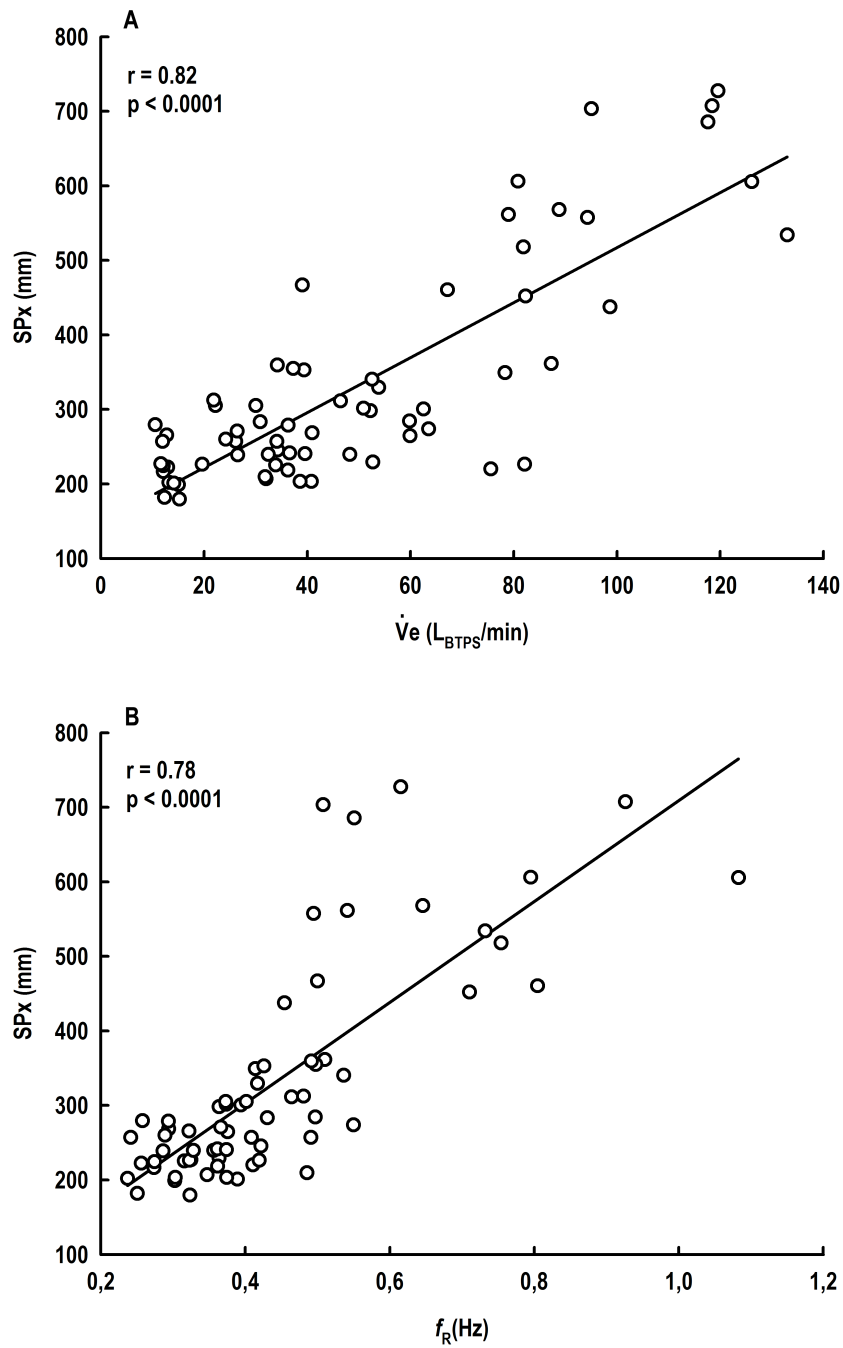


Figure 2

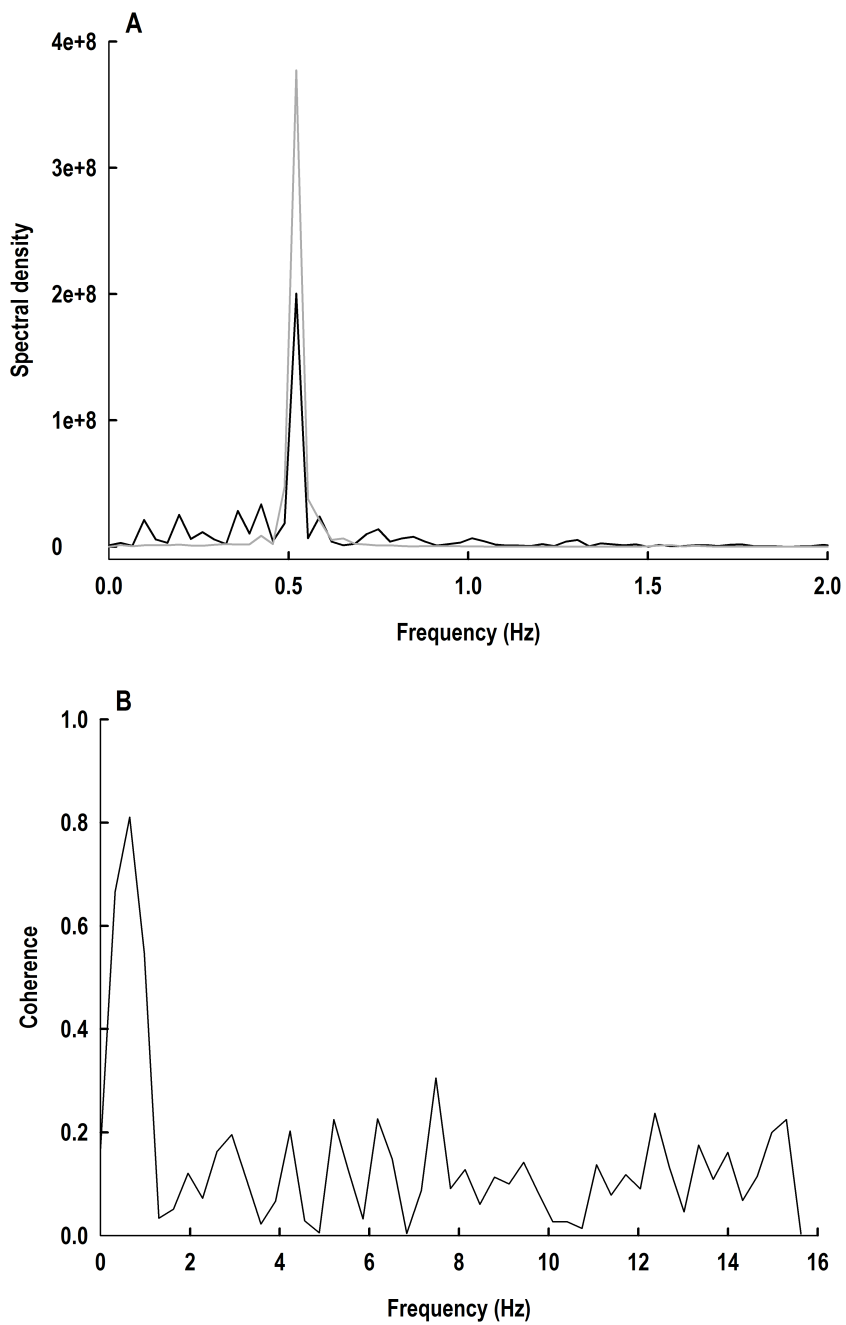


Figure 3

