Do the changes in acid-base status and respiratory gas exchange explain the voluntary termination of a test performed above the maximum lactate steady state?

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Summary

Stress tests at a constant load have been a great subject of interest for physiologists to analyze the factors which lead to voluntary termination. Several factors are responsible for voluntary termination in such efforts. The objective of this work was to study if any of the respiratory gas exchange and acid-base status variables could justify fatigue during a constant load test performed above maximum lactate steady state. Twelve amateur road cyclists performed a 30 min test on a road bicycle at an intensity of 5% above maximal lactate steady state ($MLSS_{5\%}$). Gas exchange and acid-base data were analyzed at rest and at 5, 10 and 15 min during the test. A two-way analysis of variance for repeated measures was conducted to test the effect of time and group (An alpha of 0.05 was used as the level of statistical significance for all analyses). The group that did not finished the $MLSS_{5\%}$ (N-$MLSS_{5\%}$) started from a more pronounced state of metabolic acidosis than the group that ended the test (Y-$MLSS_{5\%}$) (44,6 versus 41,7 nm/l H^+) ($F_{1,9}$ = 9.43, P = .013; η^2 = 0.51). During the test, the acid-base status was greater in the N-MLSS group than the Y-MLSS group (at 15 min, 44,3 for the Y-MLSS group and 49,2 for the N-MLSS group). Neither of the two groups showed an altered ventilation perfusion ratio, estimated by the V_D/V_T relationship, although the behaviour of PET CO_2 could suggest this outcome. A change in the breathing pattern (V_T/T_I) does not explain the termination of steady exercise in the N-MLSS group. In conclusion the results of this study do not explain the voluntary termination of exercise in a group of cyclists (N-MLSS) that made a steady effort over the maximal lactate steady state. This finding reinforces the hypothesis that fatigue occurs due to an integration of the afferent feedback of various physiological systems.

Key words:
Maximum lactate steady state. Fatigue. Acid-base status. Respiratory gas exchange.

¿Pueden los cambios del estado ácido-base e intercambio de gases respiratorios explicar el abandono de una prueba realizada por encima del máximo estado estable de lactato?

Resumen

Las pruebas de esfuerzo realizadas a intensidad constante han sido objeto de interés de los fisiólogos, analizando los factores responsables del abandono. El objetivo ha sido estudiar si variables de intercambio respiratorio y del estado ácido-base podrían justificar la fatiga durante un esfuerzo constante realizado a una intensidad superior al máximo estado estable de lactato. Doce ciclistas realizaron una prueba en su bicicleta durante 30 min a una intensidad del 5% por encima del máximo estado estable de lactato ($MLSS_{5\%}$). Se analizaron determinados parámetros de intercambio de gases respiratorios y del estado ácido-base en reposo, a los 5, 10 y 15 min de la prueba. Para determinar el efecto del tiempo y del grupo se realizó un análisis de varianza repetido de dos factores. Para valorar las posibles diferencias estadísticas se consideró un valor de alfa de 0,05. El grupo que no finalizó la prueba $MLSS_{5\%}$ (N-$MLSS_{5\%}$) comenzó con un estado de acidosis metabólica mayor que el grupo que si completó la prueba (Y-$MLSS_{5\%}$) (44,6 *versus* 41,7 nm/l H^+) ($F_{1,9}$ = 9,43, p = 0,013; η^2 = 0,51). Durante la prueba, el estado ácido-base fue mayor en el grupo N-MLSS que en el Y-MLSS (a los 15 min, 44,3 para el grupo Y-MLSS y 49,2 para el grupo N-MLSS). Ninguno de los dos grupos mostró una alteración de la relación ventilación/perfusión (relación V_D/V_T), aunque el comportamiento de la PET CO_2 podría sugerirlo. El cambio en el modelo respiratorio (V_T/T_I) tampoco explica el abandono del ejercicio estable en el grupo N-MLSS. En conclusión, los resultados de este estudio no explican el abandono del ejercicio en un grupo de ciclistas (N-MLSS) durante el esfuerzo realizado. Este hallazgo refuerza la hipótesis relativa a que la fatiga sucede debido a la integración de la retroalimentación aferente de diversos sistemas fisiológicos.

Palabras clave:
Estado de máximo estado estable de lactato. Fatiga. Estado ácido-base. Intercambio de gases respiratorios.

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Introduction

Stress tests at a constant load have been developed from two points of view: theoretical and practical. On the one hand, the interest of physiologists was focused on the physiological mechanisms that may explain fatigue as many endurance sports present a relatively constant intensity. For example, in track athletics and the triathlon, the fastest pace an athlete can sustain during an endurance event is strongly related to the rate of aerobic energy production approximating the threshold of blood lactate accumulation (LT), or the onset of hyperventilation (ventilatory threshold, VT)[1,2]. This has motivated many researchers to identify a variety of different physiological variables (*i.e.* maximum oxygen uptake, anaerobic threshold, lactate threshold, ventilatory threshold) as key predictor variables. Thus, the reasons for researchers to study steady state exercises can be summarized in two major goals: 1) to discover the physiological mechanisms that lead to fatigue when performing tests at a constant load at different intensities[3-6]; and 2) to identify the physiological factors that may predict better performances.

There are a several factors responsible for inducing local muscle fatigue, including the failure of sarcoplasmic reticulum calcium release[7], impaired sodium/potassium pump activity[3,8,9], and the slowed cross-bridge cycling[10] due to a variety of metabolic mediators including reactive oxygen species[11]. It is also clear that these muscle factors stimulate a number of neural pathways[12] that ultimately lead to reduced central motor drive and neural activation[13]. It is highly likely that many of these factors are redundant, and may be more or less prominent in leading to termination of effort under different circumstances. The link among metabolic demand, cardiovascular control (regulation of cardiac output and local muscle blood flow), neural pathways and nervous central integration (The Central Governor Model) will explain fatigue in all of its dimensions[14-17]. All efferent signals would be processed by the central command that would send a signal to all physiological systems involved or not during the exercise performance in order to protect the individual from a catastrophic failure that would force them to stop the exercise.

Based on everything noted above, the objective of this work was to study if any of the respiratory gas exchange variables and the concentration of lactate and protons could justify the fatigue that can be experienced during an exercise test at a constant load above maximum lactate steady state (MLSS). Some parameters of respiratory gas exchange, such as the ratio of the volumes of physiological dead space and tidal volume (V_D/V_T), could be modified during an exercise at a constant load. Likewise, a disturbance of acid-base status could determine a state of metabolic acidosis causing the termination of exercise. Therefore, it was hypothesized that the gas exchange and acid-base variables in MLSS+5% would explain the fatigue observed at intensities above MLSS.

Material and method

Subjects

Twelve amateur road cyclists (elite-sub23 category) were selected for this investigation (21.0 years (2.6), 179.8 cm (7.5), 72.2 kg (9.0)). The athletes had an amateur competition experience of 2.8 (1.0) years, and in particular, some of these athletes ranked among the best cyclists in Spain (i. e., top 20 in the 2008 ranking of the *"Real Federación Española de Ciclismo"* [Royal Spanish Cycling Federation] (RFEC)). A physical examination before the start of the study was used to ensure that each participant possessed a good health status. The benefits and risks of the protocol were explained and the subjects signed an informed consent form. The study obtained the approval of the ethical committee of the Technical University of Madrid.

Experimental design

Each subject carried out two constant load tests of 30 minutes corresponding to MLSS and 5% above (MLSS $_{+5\%}$). These steady state tests were carried out with a 48 h interval between tests. Each cyclist performed all tests at the same time and under similar environmental conditions (22.8 ºC (0.6) and 62.4% (4.4) relative humidity). Subjects were asked to refrain from hard physical work and consumption of any medication or stimulants for at least 24 before each experimental session.

During the tests, subjects adopted the conventional upright cycling posture. This posture is characterized by a trunk inclination of ~75º and by the subject placing their hands on the handlebars with elbows slightly bent (~10º). Before the tests, each cyclist adjusted the corresponding ergometer and used their own clip-on pedals.

Gas exchange data were collected continuously during two steady state tests using an automated breath-by-breath system (Jaeger Oxycon Pro gas analyzer, Erich Jaeger, Viasys Healthcare, Germany). The following variables were recorded during the tests: oxygen uptake (VO_2), carbon dioxide output (VCO_2), respiratory exchange ratio (RER), ventilation (VE), respiratory rate (RR), tidal volume (V_T), inspiratory time (T_I), expiratory time (T_E) and the relationships between T_I and total time (T_{TOT}) (duty cycle; T_I/T_{TOT}), V_T and T_I (V_T/T_I), V_T and T_E (V_T/T_E) and V_D and V_T (V_D/V_T). The values were averaged for a 15 s period. A 12-lead electrocardiogram (ECG; Viasys Healthcare, Germany) was continuously recorded during the tests to determine heart rate (HR).

The two steady state tests were performed on a road bicycle fitted with a SRM powermeter (Schoberer Rad Messtechnik SRM, Jülich, Germany). The bicycle was then mounted on a Tacx CycleForce Grand Excel ergometer (Technische Industrie Tacx BV, Netherlands). The Tacx CycleForce Grand Excel was not used for analysis purposes and was only used as a platform to mount the test rig on. Participants were allowed to use their own pedals and saddle. Height and reach were adjusted to match the participant's own bicycle as closely as possible.

The first constant workload trial was performed at an intensity corresponding to the mean point of VT, previously calculated by a maximal incremental test. Another 30 min test with a maximal workload (W_{max}) of 5% higher intensity was performed 48 h later if, during the first test, a steady state or a decrease of $[La^-]$ was observed (MLSS+5% intensity). Inversely, if the $[La^-]$ increased continuously or the exercise was interrupted due to the subject's fatigue during the first 30 min test, the workload was decreased by 5% W_{max}. Only two steady state tests were necessary to determine MLSS: MLSS and $MLSS_{+5\%}$ intensities. MLSS was defined as the highest workload that can be maintained with an increase in $[La^-]$ lower than 1.0 mmol/l during the final 20 min of the constant load tests[18-22]. The Borg's Scale 6-20 was used to evaluate RPE at the end of the constant workload trials[23].

Blood processing and data analysis

Before each test, an 18G catheter was inserted into a forearm vein for arterialized venous blood sampling. Arterialization was ensured by warming the forearm with an electric heating pad[24,25]. Arterialized venous blood samples were drawn prior to and during exercise at different times in order to determine the concentration of different metabolites: at the moment when maximal effort was deemed to have been reached in the incremental test and every 5 min throughout the steady state tests (0, 5, 10, 15, 20, 25 and 30 min) and at exercise termination if the test could not be maintained.

Blood samples were collected (1 ml) into pre-heparinized syringes (PICO 50, Radiometer, Copenhagen, Denmark) and analyzed immediately using a blood gas analyzer (ABL 77, Radiometer, Copenhagen, Denmark). Hydrogen ion concentration ($[H^+]$), partial pressure of carbon dioxide (PCO_2), sodium concentration ($[Na^+]$), potassium concentration ($[K^+]$), chlorine concentration ($[Cl^-]$), bicarbonate concentration ($[HCO_3^-]$), base excess (EB) and anion gap (AG) were measured. Blood lactate concentration ($[La^-]$) was analyzed by the enzymatic method (YSI 1500, Yellow Springs Instruments Co., Ohio, USA). The strong ion difference (SID) was calculated as the difference between strong cations ($[Na^+] + [K^+] + [Ca^{+2}]$) and strong anions ($[Cl^-]$ + ($[La^-]$).

Two blood samples were drawn in rapid succession at determined sample points during the constant workload trials (0, 10, 20 and 30 min). The first 1 ml sample was taken for the measurements explained above. A second 3 ml sample was collected in EDTA tubes and used for the measurement of catecholamine (epinephrine, norepinephrine and dopamine) concentrations by high-performance liquid chromatography.

Of the 12 subjects, 4 subjects completed the constant trial at $MLSS_{+5\%}$ (group Y-$MLSS_{5\%}$) and 8 did not (group N-$MLSS_{5\%}$). The gas exchange variables were averaged every 5 minutes (0, 5, 10, 15, 20, 25 and 30 min), corresponding to the determinations of the variables of acid-base status ($[La^-]$, $[H^+]$, PCO_2, $[Na^+]$, $[K^+]$, $[Cl^-]$, SID, $[HCO_3^-]$, EB and AG).

Statistical analysis

All data was reported as mean (±SD). The Shapiro-Wilk test was used to assess the normality of the data. A two-way analysis of variance for repeated measures (ANOVA) was conducted to test the effect of time (within-subject independent variable with 5 levels for acid-base variables and 4 levels for gas exchange variables) and group (between-subject independent variable: Y-$MLSS_{5\%}$ and N-$MLSS_{5\%}$ groups) on the gas exchange and acid-base dependent variables. The Bonferroni test was applied post hoc and partial η^2 was used as the effect size index. For data that violated the assumption of normality the Mann-Whitney test was applied. The effect size $\eta^2=Z^2/(N-1)$ for Mann-Whitney test was calculated[26]. Thresholds of .01, .06, and .14 for small, medium, and large effect size, respectively, were used[27]. All analyses were carried out with SPSS version 19 (Chicago, Illinois, USA). An alpha of 0.05 was used as the level of statistical significance for all analyses.

Results

Acid-base balance

The differences between the two groups in averaged acid-base parameters are presented in Table 1. At rest, the $[H^+]$ levels for the Y-$MLSS_{5\%}$ group were significantly lower than those obtained by the N-MLSS group ($F_{1,9}$ = 9.43, P = .013; η^2 = 0.51) and these differences were maintained at the different times evaluated ($F_{4,36}$ = 0,74; P = 0,572). The [SID] of the two groups were not significantly different: ($F_{1,9}$ = 0.42, P = .532).

Measured ions that determine the SID were significantly lower at rest, at the beginning and at 15 min for Na^+ in the Y-$MLSS_{5\%}$ group (Z = 2.01; P =0.024; η^2 = 0.37 at rest, Z = 2.40; P =0.008; η^2 = 0.52 at the beginning; Z = 2.18; P = 0.012; η^2 = 0.37 at 15 min), for K+ significantly higher at the beginning and 15 min for the group N-$MLSS_{5\%}$ (Z = 2.64; P = 0.002; η^2 = 0.63 at the beginning; Z = 2.08: P =0.021; η^2 = 0.39 at 15 min), significantly lower at the beginning for the Ca^{2+} (Z = 2.72; P = 0.003; η^2 = 0.67) for the N-$MLSS_{5\%}$ group and showed no differences for the Cl^- ($F_{1,9}$ = 0.27, P = .616) and for the HCO_3^- ($F_{1,9}$ = 0.27, P = .616) between the two groups studied. Finally, the plasma L- showed no significant difference ($F_{1,9}$ = 0.27, P = .617) between the two groups studied. AG levels in the group that completed the test were not significantly different to those obtained by the group that did not complete it ($F_{1,9}$ = 2.64, P = .139).

For both groups, the test carried out did not correspond to a maximum steady state test[10] because lactate concentration exceeded 1 mmol /L at 20 min during the constant load tests: Y-MLSS 2.8 (0.5) and 4.8 (1.3) and N-MLSS 3.3 (1.1) and 5.2 (2.3), for 5 to 15 minutes. The $PpCO_2$ did not show significant differences ($F_{1,9}$ = 1.49; P = .253) between the two

Table 1. Differences between two groups in averaged acid-base parameters during a test 5% above maximal lactate steady state.

	Y $MLSS_{5\%}$ (N = 4)				N $MLSS_{5\%}$ (N = 11)			
Time	$[H^+]$ (nmol/l)	[SID] (mmol/l)	AG (mmol/l)	$[HCO_3^-]$	$[H^+]$ (nmol/l)	[SID] (mmol/l)	AG (mmol/l)	$[HCO_3^-]$
rest	41,7 (±2,4)	34,4 (±2,1)	8,0 (±1,2)	26,5 (±1,7)	44,6 (±2,1)	33,6 (±2)	7,8 (±1,2)	25,9 (±3)
0	39,4 (±1,6)	32,3 (±1,5)	8,4 (±1,6)	23,7± (3,2)	43,5 (±2)	33,2 (±1,6)	9 (±1,9)	24,2 (±2,2)
5	43,5 (±2,8)	31,7 (±2,7)	13 (±1,5)	20,6 (±3,1)	45,7 (±2,2)	32,6 (±0,9)	13,7143 (±1,6)	21 (±0,8)
10	43,8 (±4,1)	31 (±1,3)	14,3 (±2)	19,7 (±2,4)	47,7 (±2,1)	30,9 (±2)	16,6 (±1,7)	17,6 (±2)
15	44,3 (±4)	28,6 (±2,4)	15,1 (±2,1)	17,4 (±2,8)	49,2 (±2,8)	30,3 (±2,9)	17,7 (±2,4)	16,7 (±2,9)

groups studied. Although, the lactate concentration of the Y-$MLSS_{5\%}$ group was not significantly different from the N-$MLSS_{5\%}$ group ($F_{2,36}$ = 0.12; P = .771), they were higher.

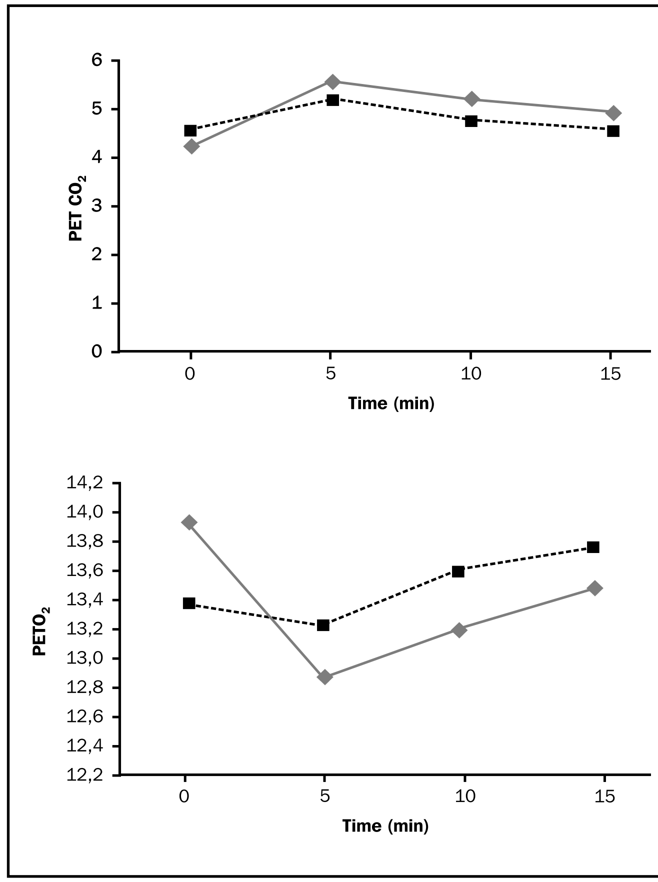
Gas Exchange variables

The change in the breathing pattern (V_T/T_i and V_T/T_e) could be due to central and peripheral factors (Clark and von Euler, 1972, Dempsey *et al*., 1986) that may be responsible for the termination of the test by the N-MLSS cyclists group. However, in the present study, V_T/T_i values, a representative central generator respiratory parameter, showed no significant differences between the two groups, rejecting the hypothesis of a change in the breathing pattern to explain the termination of steady state exercise, although ventilation at 5, 10 and 15 min was not significantly higher in the N-MLSS group.

Table 2 shows the results obtained in gas exchange data averaged variables. The averaged VO_2, VCO_2, HR and VE, showed no significant differences between the two groups: VO_2 (in L/min $F_{1,9}$ = .33; P = .580; in ml/min/Kg $F_{1,9}$ = .01; P = .933); VCO_2 ($F_{1,9}$ = 0.45; P = .522); HR ($F_{1,9}$ = .21; P = .655); V_E ($F_{1,9}$ = 0.24; P = .638). At 5, 10 and 15 minutes the PET CO_2 of the Y-$MLSS_{5\%}$ group showed higher values that of the N-$MLSS_{5\%}$ group (Figure 1a; $F_{1,12}$ = 3.53; P = .034; η^2 = 0.27;). PET O_2 resting values of the Y-$MLSS_{5\%}$ group were higher than those of the N-$MLSS_{5\%}$ group, but no significant differences between groups were observed at 5, 10 and 15 min ($F_{2,13}$ = 6.74; P = .002; η^2 = 0.43; Figure 1b).

Indirect parameters of ventilation/perfusion ratio (V_D/V_T) and respiratory pattern (V_T/T_i, V_T/T_e) response during the constant workload trials are represented in Figure 2. No differences were demonstrated between the two groups for V_D/V_T ($F_{1,9}$ = 0.02; P = .906) and V_T/T_i ($F_{1,8}$ = 0.01; P = .960).

Figure 1. PET CO_2 (side a) and PO_2 (side b) data for two groups at rest and during a test 5% above maximal lactate steady state. In solid line the Y-MLSS group and broken line the N-MLSS group.



Discussion

The main finding of this study demonstrated the physiological reasons that explain exercise termination or fatigue at 5% intensity above maximal steady state lactate (>5% MLSS).

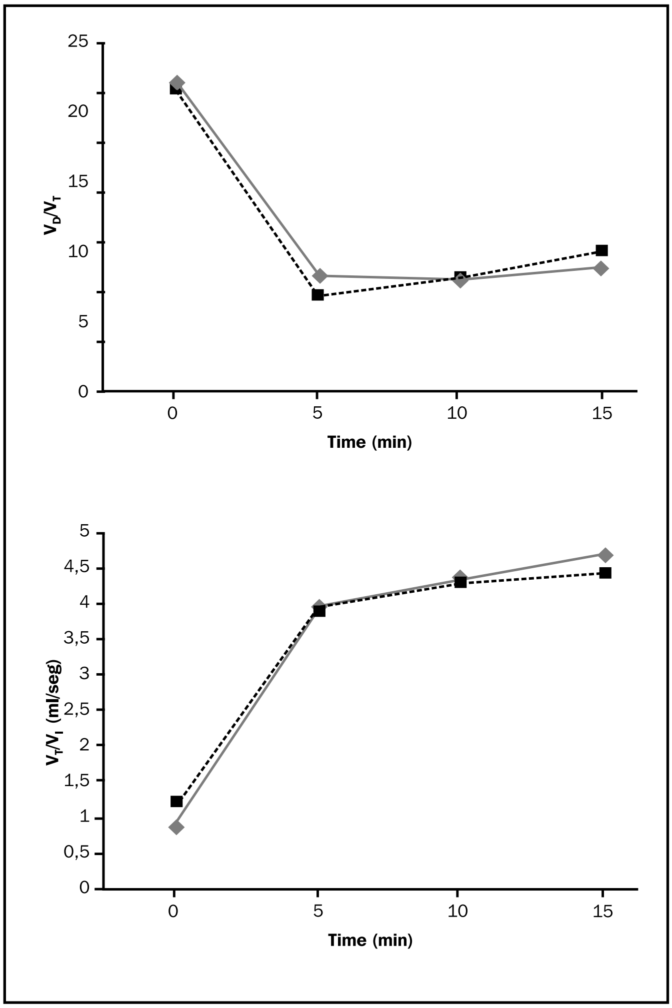
Firstly, it is noteworthy that the N-MLSS group started from a more pronounced state of metabolic acidosis. This might indicate that the rest time between the two stable tests (48 hours) was insufficient for cyclists from the N-MLSS group. It is possible that the recovery time after intense exercise could affect the muscular acid status, conditioning the next steady state test[28]. As noted by McKelvie *et al*[29], it is possible that the recovery time for the N-MLSS group was not enough for renal function to compensate for the acid load during the test made 48 hours before.

Secondly, the acid-base status was greater in the N-MLSS group than in the Y-MLSS group. For example, at 15 minutes, the [H^+] was 44.3 nmol/l and 49.2 nmol/l for the Y-MLSS and the N-MLSS groups respectively. That is, both groups had an acute metabolic acidosis, but it was significantly higher for the N-MLSS group. However, the [SID] in the 15th minute was lower for the Y-MLSS group. While we have mea-

Table 2. Gas exchange data averaged variables during a test 5% above maximal lactate steady state.

Time	Y $MLSS_{5\%}$ (N = 4)				N $MLSS_{5\%}$ (N = 11)			
	VO_2 (ml/min)	VCO_2 (ml/min)	HR (beats/min)	V_E (L/min)	VO_2 (ml/min)	VCO_2 (ml/min)	HR (beats/min)	V_E (L/min)
rest	517,7 (±71,2)	470,7 (±94,9)	55 (±11)	16 (±3)	443,7 (±146,7)	378,6 (±132,7)	60 (±14)	14 (±4)
5	4267,8 (±410,1)	4134,6 (±310,4)	165 (±16)	106 (±7)	4147,9 (±511,3)	4029,1 (±576,8)	164 (±7)	111 (±17)
10	4474,7 (±436,8)	4257 (±210)	171 (±9)	118 (±6)	4401,9 (±468,8)	4143,4 (±460,5)	174 (±7)	126 (±16,96479)
15	4568,1 (±446,2)	4279,7 (±181,7)	175 (± 9)	127 (±8)	4316,2 (±520,2)	4013,4 (±534,5)	179(±7)	130 (±17)

Figure 2. V_D/V_T (side a) and V_T/T_i (side b) ratios for two groups at rest and during a test 5% above maximal lactate steady state. For V_D/V_T ratio, in solid line the N-MLSS and broken line the Y-MLSS group. For V_T/T_i, solid line the Y-MLSS group and broken line the N-MLSS group.



sured the actual SID[1], not considering plasma ions that can affect this measure[7,30]. The greatest value of the SID in the N-MLSS group confirms the heightened state of acidosis at the end, since it is considered that effective SID is lower by about 2 mEq/l than apparent SID[2].

The highest value of the SID in the N-MLSS group may have several explanations. More [K+] and lower [Na+] in extracellular fluid in the Y-MLSS group at 5, 10 and 15 min could explain the mechanism of fatigue, as was suggested by Lidinger[8,9]. These variations of ionic plasma concentration could be the result of a "burnout" of the sodium / potassium pump in muscle cells which would determine an increase in intracellular sodium and a decrease in the intracellular fluid. Moreover, the displacement of water occurs from the interstitial fluid into the cell during exercise[31] which could cause a variation of the concentration of total anions and then the SID[11] .

The change in the breathing pattern (V_T/T_i and V_T/T_e) could be due to central and peripheral factors that may be responsible for the abandonment of the N-MLSS cyclists group. However, in the present study, V_T/T_i values, representative of the respiratory central generator parameter[28], showed no significant differences between the two groups, rejecting the hypothesis of a change in the breathing pattern to explain the termination of steady exercise, although ventilation at 5, 10 and 15 min was not significantly higher in the N-MLSS group. The VE increase in the N-MLSS group probably reflects a decreasing contractile capacity of the inspiratory muscles when experiencing a continuous stable load[32].

The V_D/V_T ratio is a parameter that can indicate indirectly changes in the ventilation/perfusion relationship[33]. Neither of the two groups (Y-MLSS and N-MLSS) shows an altered ventilation perfusion ratio (from the point of view of gas exchange) (Figure 1). By contrast, the tendency of the V_D/V_T ratio is an adaptation to the increased demand produced during the stable test, with no significant differences between the two groups. A limitation of this study was to not evaluate the changes in PaO_2, P_AO_2, and $P(A–a)O_2$ that could prove hypothetical arterial hypoxemia. Despite following the Foster methodology[34] the results of the arterialized venous blood were not acceptable for analysis. Therefore, the result allows us to reject the hypothesis that a possible modification of the ventilation perfusion ratio caused the N-MLSS group to terminate the test.

However, although no differences were observed in the V_D/V_T relationship, the interaction effect between time and group of cyclists ($F_{1,12} = 3{,}53$; $P = 0{,}034$; $\eta^2 = 0{,}27$) for PET CO_2 is noteworthy. While PET CO_2 resting values of the Y-MLSS group were lower than the N-MLSS group, at 5, 10 and 15 minutes, the Y-MLSS group showed higher values (Figure 2a). By contrast, for PET O_2 (interaction effect $F_{2,13} = 6.74$; $P = .002$; $\eta^2 = 0.43$) no significant differences were found between groups at 5, 10 and 15 minutes during the $MLSS_{5\%}$ (Figure 2b). Due to the behaviour of PET CO_2 it is interesting to point out that the N-MLSS group tended towards an alteration of the ventilation/perfusion relationship, reflecting insufficient removal of carbon dioxide (lower values of PET CO_2). However, because the results of the arterialized venous blood were inadequate it cannot be thought that a change in ventilation perfusion ratio for PET CO_2 values could justify the termination of the test by the N-MLSS group.

In summary, the results of this study do not explain the voluntary termination of exercise in a group of cyclists (N-MLSS) that made a steady effort over the maximal lactate steady state and are not in agreement with our initial hypothesis, because breathing pattern and acid-base variables did not show a different response compared with the Y-MLSS group. The differences found in the acid-base status between the two groups are not sufficient to explain the termination of the exercise. In addition, the results of the trends in the respiratory exchange variables studied do not suggest a modification of the ventilation/perfusion ratio in the N-MLSS group. Our results are in accordance with previous studies and reinforce the hypothesis that fatigue occurs due to an integration of the afferent feedback of various physiological systems[35].

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