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Do 5% changes around maximal lactate steady state lead to swimming biophysical modifications?

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ABSTRACT

Our purpose was to examine the swimming biophysical responses at velocities (v) of 97.5, 100 and 102.5% of the maximal lactate steady state (MLSS). Ten elite female swimmers performed three-to-five 30-min constant tests at imposed paces to determine 97.5, 100 and 102.5%MLSS v. Gas exchange, blood lactate concentration ([La-]), stroke rate (SR) and v were determined during each test. The v values at 97.5, 100 and 102.5%MLSS were 1.21 ± 0.07, 1.24 ± 0.07 and 1.27 ± 0.07 m.s^{-1}, respectively. Oxygen uptake ($\dot{V}O_2$) and Pulmonary ventilation ($\dot{V}E$) increased as function of v. SR and stroke length (v/SR = SL) increased as a function of v. All measured variables were constant as a function of time at 97.5%MLSS and 100%MLSS. At 102.5%MLSS SR increased (3.5%) and stroke length (SL) decreased (3.5%) as a function of time. While $\dot{V}O_2$ was constant at 102.5%MLSS, [La-] and $\dot{V}E$ increased as a function of time, suggesting hyperventilation, at v's of 97.5%MLSS and 100%MLSS swimmers completed the 30 min swim in spite of decreased SL and increased SR. However, the decrease in SL and increased SF were accompanied by increased [La-] and $\dot{V}E$ and resulted in the inability of most swimmers to complete the 30 min swim presumably due to fatigue at 102.5%MLSS.

1. Introduction

At low exercise intensities metabolism is from primarily aerobic sources. However, when exercise intensity increases to near or above maximal aerobic power a mixture of aerobic and anaerobic sources are used, leading to time dependent increase in muscle and blood lactate. Since the 1960s, researchers have struggled to understand and define the physiologic state where there is a significant increase in blood lactate concentration ([La-]) (anaerobic threshold – AnT). Of these attempts, a recent initial definition was termed endurance performance limit (Hollmann, 2001), which has been redefined as aerobic-anaerobic threshold, individual anaerobic threshold, anaerobic threshold, lactate turnpoint, and individual lactate minimum, among other terms (Faude, Kindermann, & Meyer, 2009). Another concept related the AnT to the maximal

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intensity that can be maintained as function of time without blood lactate accumulation, i.e. the maximal lactate steady state – MLSS (Beneke, 2003). This exercise intensity has been used for the evaluation of aerobic capacity for endurance performance and training prescription (Beneke & von Duvillard, 1996; Faude et al., 2009).

The MLSS is considered by many as the direct method for the evaluation of aerobic capacity (Beneke, 2003; Beneke & von Duvillard, 1996; Faude et al., 2009). MLSS is identified as the highest steady state [La-] that can be maintained during prolonged sub-maximal and constant workload exercise (Beneke, 2003; Beneke & von Duvillard, 1996). At intensities below and at the MLSS there is a steady-state of [La-] as function of time and exercise can be sustained (Baron, Dekerle, Depretz, Lefevre, & Pelayo, 2005). However, when the exercise is performed at intensities above the MLSS intensity, a significant increase in [La-] is observed as function of time, which is associated with voluntary exhaustion (Beneke & von Duvillard, 1996; Heck et al., 1985).

Although the concepts of AnT and MLSS have been previously used to characterize swimming performance, it is commonly accepted that there are bioenergetical and biomechanical factors that may influence these parameters. Further examination of these factors is needed to better understand their possible interaction, helping to understand the swimmers' adjustments that occur at intensities around MLSS (Faude et al., 2009) and as a function of swimming time.

The inability to maintain a predetermined swimming intensity (fatigue) may be due to the inability to sustain optimal biomechanical parameters, as the aerobic system bioenergetics has been shown to be stable as a function of time (Baron et al., 2005). Physiological mechanisms other than metabolism may be time-dependent, such as the ability to sustain force and its application to the water (Baron et al., 2005; Dekerle, Nesi et al., 2005; Pelarigo, Denadai, & Greco, 2011). Thus, biomechanical factors could change the MLSS swimming velocity (v) (Pelarigo et al., 2011), leading to a reduced v or swim time.

We are unaware of any studies that have evaluated bioenergetical and biomechanical factors at intensities at or around the 100%MLSS in swimming. To examine the interrelationships of biophysical factors they must be evaluated not only as a function of intensity, but also as a function of exercise duration to understand what limits performance at these v's. Thus, the purpose of this study was to analyze the responses of bioenergetical and biomechanical factors while swimming at 97.5, 100 and 102.5%MLSS. We hypothesized that swimming intensities up to 100%MLSS would not require progressive adjustments of bioenergetical and biomechanical factors and not limit exercise endurance up to 30 min. However, we hypothesized that swimming above 100%MLSS would compromise bioenergetical and biomechanical factors which would affect the swimmers ability to sustain set exercise intensities (v) for 30 min.

2. Methods

Ten elite female swimmers (mean ± SD; aged 17.6 ± 1.9 years, height 1.70 ± 0.05 m, body mass 61.3 ± 5.8 kg and percentage of body fat mass 15.5 ± 2.9%; maximal oxygen uptake – $\dot{V}O_{2max}$ 54.9 ± 6.7 mL.kg.min^{-1}), who specialized in middle- and long-distance swimming events participated in the present study. The measurements of body mass and fat were assessed by a segmental body composition analyzer (Tanita, TBF 305, Tokyo, Japan).

Subjects had, at the least, seven years of experience as competitive swimmers and their mean performance over the 400 m freestyle swim was 88.0 ± 3.4% of the 2016 short course world record. The study was approved by the local ethics committee and was performed according to the Declaration of Helsinki. Subjects and/or parents gave their written informed consent before participation in experiments.

The test sessions were performed in a 25 m indoor swimming pool, with water temperature of 27–28 °C and air humidity of 40–60%. Swimmers were advised to refrain from intense training for at least 24 h before the experiments. The tests were all conducted within a seven day period, at the same time of the day (±2 h) to minimize the effect of circadian rhythm. In all test sessions, the swimmers performed a 1000 m warm-up at low/moderate aerobic intensity. During the tests, swimmers swam front crawl and used in-water starts and open turns without underwater glides.

First, the swimmers performed an intermittent progressive protocol until voluntary exhaustion to determine the individual anaerobic threshold (IAnT). The predetermined initial v of the swim was set at ~80% of the subject's best time for the 400 m front crawl race (S400), the v was increased by 0.05 m.s^{-1} for each subsequent step until voluntary exhaustion. Thirty seconds rest intervals were observed in-between each swim. The distance of each step of the incremental test was 200 m.

Earlobe capillary blood samples (5 μL) were collected and analyzed for [La-] with a portable lactate analyzer (Lactate Pro, Arkray, Inc., Kyoto, Japan). [La-] was measured at rest and in the first 30 s after each step of the incremental test and, immediately after exhaustion and at each 2 min of recovery from the last step until the [La-] peak was found. The IAnT was assessed by the relationship between [La-] and v with the lactate inflexion point determined as the interception between a linear and exponential regressions to estimate the v where [La-] increased exponentially (Fernandes et al., 2006; Machado, Almeida, Morais, Fernandes, & Vilas-Boas, 2006). If a swimmer did not achieve their maximal v and/or exhaustion with the pre-defined increases in v, the fastest v the subject completed was used to determine the minimum v eliciting the $\dot{V}O_{2max}$.

After determining IAnT, each swimmer performed three-to-five 30 min submaximal constant swimming tests at imposed paces to assess the v where a MLSS was achieved and maintained (100%MLSS). The swimming v was set and maintained using a visual underwater pacer (GBK-Pacer, GBK Electronics, Aveiro, Portugal), with a light strip on the bottom of the pool. The light strip had lights located 2.5 m apart for 25 m. The swimmers followed the flashing lights to maintain the predetermined v's. The swimmers were instructed to swim at a speed by looking at and following the visual signal as the lights

proceeded along the pool length. Exhaustion was defined and the test finalized when the swimmers remained 5 m behind the lights. A 24 h interval was imposed between all the tests.

[La-] was determined at rest, and at the 10th and 30th min (or voluntary exhaustion) of each continuous test as described above. The first trial was performed at the IAnT v, and, if during the first trial a steady state or a decrease in [La-] was observed, further subsequent trials with 2.5% higher v were performed until no [La-] steady state was observed. If the first trial resulted in a clearly identifiable increase of the [La-] and/or could not be sustained due to exhaustion, further trials were conducted with subsequently reduced v's (Pelarigo et al., 2011). The MLSS was defined as the highest [La-] that increased by no more than 1 mmol.L^{-1} between the 10th and the 30th min of the test (Heck et al., 1985). The corresponding [La-] value was assumed to be the average of the value at the 10th and 30th min of exercise.

Bioenergetical factors were divided into two categories: oxygen uptake ($\dot{V}O_2$) and total energy expenditure ($\dot{E}$). The latter was the product of $\dot{V}O_2$ and caloric equivalent estimated from respiratory exchange ratio. The energy cost of swimming (C) per unit distance was calculated from $\dot{E}$ and v ($\dot{E}/v = C$) and was termed economy. $\dot{V}O_2$ was calculated from measures of expired ventilation ($\dot{V}E$) and O_2 and CO_2 fractions by a telemetric portable analyzer (K4b^2, Cosmed, Italy), using standard equations. The end-tidal CO_2 (ETP_aCO_2) was used as an estimate of the arterial CO_2. The analyzer was connected to the swimmer by a low hydrodynamic resistance respiratory snorkel and valve system (New AquaTrainer®, Cosmed, Italy). This system has been previously validated and used in similar studies (Baldari et al., 2013; Sousa, Vilas-Boas, & Fernandes, 2014). The equipment was calibrated prior to each swim for $\dot{V}E$ with a calibrated syringe and the O_2 and CO_2 analyzers with standard calibration gases. The values of gas exchange were measured breath-by-breath during all the tests and averaged every 5 s (Fernandes et al., 2012). The ratio of $\dot{V}O_2$ and $\dot{V}E$ was calculated and termed oxygen uptake efficiency (OUE) for all conditions (Baba et al., 1996). Heart rate (HR) was monitored and registered continuously by a HR monitor system (Polar Vantage NV, Polar electro Oy, Kempele, Finland) and transferred telemetrically to the K4b^2 device. The values of HR were also averaged for 5 s intervals.

The energetic parameters were $\dot{E}$ and C. The C was determined using the caloric equivalent of the $\dot{V}O_2$ (kcal.L^{-1} O_2) calculated by the respiratory exchange quotient (Fletcher, Esau, & Macintosh, 2009). The C was calculated using the Eq. (1):

$$C = \dot{V}O_2 \text{ caloric equivalent} \cdot v^{-1} \quad (1)$$

where C is energy cost of locomotion calculated by indirect calorimetry expressed as kJ.m.$^{-1}$, $\dot{V}O_2$ was measured in L.min^{-1}, caloric equivalent is in kcal.L^{-1}, and v is velocity in m.min^{-1}. The energy equivalent was converted to SI units, where 1 kcal is equivalent to 4.184 kJ.

$$\dot{E}.kg^{-1} = C \cdot v \quad (2)$$

The $\dot{E}$ was determined by the product of C and v, corrected by the body mass, where $\dot{E}$ is the total energy expenditure expressed as mL.kg^{-1}.min^{-1}, C as kJ.m^{-1}, v is velocity in m.min^{-1}, body mass is in kg. The energy equivalents were converted into the SI units according to di Prampero (1986) where 1 mL O_2 is equivalent to 20.9 J.

Biomechanical factors measured were stroke rate (SR), stroke length (SL) and the product of v and SL (stroke index – SI) at each v studied. The biomechanical analysis was conducted during the pure swimming phase of each lap in the middle of the pool (between 7.5 and 17.5 m). SR was determined from images of an above-water video camera (DCR-HC42E, Sony, Japan) operating at a frequency of 50 Hz. The SR and SL were determined from two limbs cycles for each condition and the data were averaged. The video data were then used to calculate v (m.s^{-1}) and SR (cycles.min^{-1}). SR was determined by the number of upper limb cycles per unit of time and SL was calculated by the ratio of v and SR.

To normalize for differences in v and time for each subject, the data for the continuous tests at 97.5, 100 and 102.5%MLSS v's were normalized to the total time of each swim (100%). The data were split into eight time points corresponding to rest, the initial steady state determined during the 4th min of the swim and then 25, 33, 50, 66, 75 and 100% of the total duration of exercise.

The data are presented as mean and standard deviation (±SD). Normality and sphericity of data were checked with the Shapiro-Wilk's W and Mauchley tests. When the assumption was not attained, Greenhouse-Geisser or the Huynh-Feld adjusted univariate tests for repeated measures were used. Beyond descriptive statistics, the analyses of $\dot{V}O_2$, $\dot{V}E$ and C and SR, SL and SI were performed using multivariate ANOVA. The analysis of [La-] and v values were performed using the univariate ANOVA. All analyses were repeated measures ANOVAs, complemented with the Tukey correction post hoc test. The significance level of $p < 0.05$ was used for all comparisons.

3. Results

The results of the experiments are presented for the v's at 97.5, 100 and 102.5% MLSS intensities. For each v, data at rest, at the 4th min, and at 25, 33, 50, 66, 75 and 100% of swimming time are also shown. The average v values for the three conditions were different from each other, with 97.5%MLSS slowest and 102.5%MLSS fastest (Fig. 1). All swimmers completed the 30 min swims at 97.5 and 100%MLSS. However, eight out of the ten swimmers were not able to maintain the predetermined v during the 30 min of the 102.5%MLSS swim, reaching voluntary exhaustion on average at 19.3 ± 4.9 min of exercise. Regarding the statistical hypothesis decomposition, the interaction effect, i.e., significance level ($p < 0.05$) between the

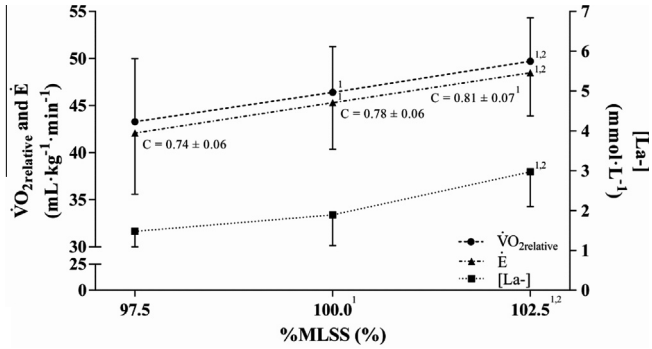

Fig. 1. Mean ± SEMI-SD of oxygen uptake corrected for body mass ($\dot{V}O_{2relative}$), total energy expenditure ($\dot{E}$), energy cost of locomotion (C) and blood lactate concentration ([La-]) values at velocities corresponding to 97.5, 100 and 102.5% (slowest to highest velocity, respectively) of the maximal lactate steady state (MLSS) plotted as a function of velocity. [1,2] Values significantly different to 97.5 and 100%MLSS, respectively ($p < 0.05$).

intensity and its time effects, only occurred with the $\dot{V}E$ and OUE, in which the remaining bioenergetical and biomechanical variables achieved an intensity and/or time effect.

Fig. 1 presents $\dot{V}O_2$, $\dot{E}$, C and [La-] values as a function of v during the three swimming conditions. Oxygen uptake corrected for body mass ($\dot{V}O_{2relative}$) and $\dot{E}$ increased significantly as a function of v throughout the studied intensities. C increased as a function of v between 97.5 and 102.5%MLSS. [La-] values were similar at 97.5 and 100%MLSS, however, the values were higher at 102.5%MLSS. Furthermore, the [La-] values as a function of time were similar at the 10th min and 30th min (or voluntary exhaustion) for 97.5 (1.4 ± 0.3 and 1.6 ± 0.5 mmol.L^{-1}), 100 (1.9 ± 0.8 and 1.9 ± 0.7 mmol.L^{-1}) and 102.5%MLSS (2.8 ± 0.9 and 3.1 ± 1.0 mmol.L^{-1}), respectively.

The bioenergetical and biomechanical parameters are presented in Table 1. The resting values of all parameters were not different among the three swimming conditions. As expected, the $\dot{V}O_2$, and $\dot{E}$ increased significantly as a function of v. C increased as function of v between 97.5 and 102.5%MLSS. $\dot{V}E$ increased significantly as a function of v's at 97.5, 100 and 102.5%MLSS. The HR values increased as a function of v and were significantly higher at 102.5%MLSS compared to 97.5% MLSS. The OUE values were lower at the 4th min and 50, 66, 75 and 100% of swim time at 102.5%MLSS than at 97.5%MLSS.

$\dot{V}O_2$ did not significantly change as a function of time for v's at 97.5, 100 and 102.5%MLSS and averaged 2.62 ± 0.04, 2.82 ± 0.04 and 3.03 ± 0.02 L.min^{-1}, respectively. Similarly $\dot{E}$ and C did not significantly change as a function of time for v's at 97.5, 100 and 102.5%MLSS. $\dot{V}E$ did not significantly change as a function of time for v's at 97.5 and 100%MLSS and averaged 63.94 ± 1.29 and 69.73 ± 1.15 L.min^{-1}, respectively. However at 102.5%MLSS, $\dot{V}E$ increased significantly as function of time from 75.7 ± 6.7 L.min^{-1} at 4th min to 82.9 ± 9.4 L.min^{-1} at 100% of swim time (hyperventilation). The differences for $\dot{V}E$ described above have to acknowledge that there was a statistical interaction between swimming v (97.5, 100 and 102.5% MLSS) and the time points (4th min, 25, 33, 50, 66, 75 and 100%) of measurements and for OUE.

ETP_aCO_2 values were similar at all v's, at 102.5%MLSS, however, it showed a tendency to be lower (mean = 37.60 mmHg) than at the two lower v's (97.5%MLSS – mean = 38.92 mmHg; p = 0.103 and 100%MLSS – mean = 38.99 mmHg; p = 0.083) and what is considered normal P_aCO_2 (40 mmHg). Furthermore, the ETP_aCO_2 values decreased as a function of time in the 102.5% MLSS swim from the start of the exercise (4th min) to its end for all swimming speeds ($F_{6,54} = 10.328$, $p < 0.001$, $\eta_p^2 = 0.499$).

Regarding the time effect, the $\dot{V}O_2$ values were lower at the 50, 66 and 100% compared to 4th min, and at the 50% compared to 25% of exercise. Subsequent to the decreased $\dot{V}O_2$, C and $\dot{E}$ were lower at the 50% time compared to the 4th min and the 25% time of exercise. The HR values increased at the all swim times compared to the 4th min, as well as at 66, 75 and 100% of exercise compared to the 25%, at 75% and 100% comparing 33%, and comparing 100% than at 50% time point.

OUE decreased as a function of time at the 66, 75 and 100% of the v at 97.5%MLSS compared to the 4th min of exercise at 97.5%MLSS. For the v at 100%MLSS, OUE was significantly lower at 66% of swim time compared to the 4th min. For the v at 102.5%MLSS, OUE was significantly lower at 66, 75 and 100% of swim time compared to the 4th min and 25 and 33% of swim time exercise, and 100% of swim time compared to the 50%.

Fig. 2 presents the changes in SR, SL and SI values as a function of v during the three swimming intensities. SR values increased as a function of v for all intensities. SL was lower at 102.5%MLSS compared to 97.5 and 100%MLSS, 4% on average across exercise times. The SI values were not significantly different among the three v's and averaged 2.61 ± 0.02, 2.68 ± 0.03 and 2.65 ± 0.05 m^2.s^{-1}cycle^{-1}, respectively.

SR's during all swims were not significantly different up to 50% of exercise time at all v's. SR's significantly increased above 50% exercise time up to 100% of exercise time at each v (3–5%). During the 30 min swim, SL and SI were lower at 75% and 100% MLSS time compared to the 4th min of exercise, and at 100% compared to the 33% and 50%MLSS of exercise time.

4. Discussion

This study analyzed the effects of intensity and time-dependent variation of bioenergetical and biomechanical factors in swimming at intensities below, at, and above the MLSS. The MLSS is considered a commonly accepted method for the

Table 1
Bioenergetical and biomechanical parameters associated with the velocity at 97.5, 100 and 102.5% of the maximal lactate steady state (MLSS) for the 8 normalized time moments of the swims. Statistical analyses were described by intensity and/or time effect or interaction effect (N = 10).

Parameters/Effect	Intensity	Time Moments							
		Rest	4th min	25%	33%	50%	66%	75%	100%
$\dot{V}O_2$[#*] (L·min^{-1})	97.5%MLSS	0.44 (0.15)	2.68 (0.28)	2.63 (0.29)	2.61 (0.28)	2.58 (0.28)^{a,b}	2.59 (0.30)^{a}	2.65 (0.28)	2.63 (0.28)^{a}
	100%MLSS	0.36 (0.05)	2.88 (0.23)^{1}	2.88 (0.22)^{1}	2.81 (0.21)^{1}	2.78 (0.19)^{1,a,b}	2.80 (0.22)^{1,a}	2.81 (0.17)^{1}	2.80 (0.21)^{1,a}
	102.5%MLSS	0.39 (0.06)	3.05 (0.21)^{1,2}	3.05 (0.19)^{1,2}	3.06 (0.22)^{1,2}	3.02 (0.21)^{1,2,a,b}	3.00 (0.22)^{1,2,a}	3.03 (0.21)^{1,2}	3.01(0.20)^{1,2,a}
$\dot{V}E$[†] (L·min^{-1})	97.5%MLSS	14.7 (5.0)	62.4 (8.1)	63.6 (9.6)	63.3 (8.7)	62.9 (8.9)	64.7 (9.5)	66.2 (10.0)	64.5 (9.3)
	100%MLSS	12.1 (1.8)	68.6 (8.2)^{1}	70.7 (8.2)^{1}	69.1 (8.4)^{1}	68.4 (7.1)^{1}	71.5 (7.6)^{1}	69.5 (8.3)	70.3 (10.5)^{1}
	102.5%MLSS	14.6 (4.5)	75.7 (6.7)^{1,2}	76.3 (5.8)^{1,2}	76.5 (7.2)^{1,2}	78.4 (9.6)^{1,2}	79.8 (8.6)^{1,2}	80.5 (9.3)^{1,2}	82.9 (9.4)^{1,2,a,b,c}
OUE[†] (mL O_2·L^{-1} $\dot{V}E$)	97.5%MLSS	31.0 (5.7)	43.3 (3.5)	41.8 (4.2)	41.5 (3.8)	41.3 (3.5)	40.4 (3.2)^{a}	40.4 (4.1)^{a}	41.1 (4.2)^{a}
	100%MLSS	30.0 (2.9)	42.3 (3.2)	41.0 (3.5)	41.1 (3.7)	40.9 (4.3)	39.6 (4.7)^{a}	40.9 (4.3)	40.4 (4.8)
	102.5%MLSS	28.3 (5.8)	40.5 (3.3)^{1}	40.2 (3.2)	40.2 (3.0)	38.8 (4.1)^{1}	37.9 (3.9)^{1,a,b,c}	38.0 (4.0)^{1,2,a,b,c}	36.6 (4.2)^{1,2,a,b,c,d}
HR[#*] (bpm)	97.5%MLSS	77.8 (11.7)	160.4 (16.3)	165.2 (15.9) ^{a}	166.6 (14.9) ^{a}	168.4 (15.4) ^{a}	168.4 (16.2)^{a,b}	170.4 (14.7)^{a,b,c}	170.6 (14.7)^{a,b,c,d}
	100%MLSS	73.0 (11.5)	168.9 (7.7)	173.3 (9.8) ^{a}	173.8 (11.2) ^{a}	173.0 (9.6) ^{a}	174.7 (10.2)^{a,b}	175.0 (10.9)^{a,b,c}	176.3 (10.5)^{a,b,c,d}
	102.5%MLSS	74.1 (11.6)	173.0 (8.8)^{1}	176.8 (10.0)^{1,a}	178.8 (10.2)^{1va}	180.3 (9.7)^{1,a}	181.7 (9.3)^{1,a,b}	182.1 (9.2)^{1,a,b,c}	182.2 (11.1)^{1,a,b,c,d}
C[#*] (kJ·m^{-1})	97.5%MLSS	0.17 (0.04)	0.75 (0.06)	0.74 (0.07)	0.73 (0.06)	0.72 (0.05)^{a,b}	0.73 (0.06)	0.74 (0.06)	0.73 (0.06)
	100%MLSS	0.16 (0.04)	0.79 (0.06)	0.79 (0.06)	0.77 (0.06)	0.76 (0.06)^{a,b}	0.77 (0.07)	0.77 (0.06)	0.77 (0.07)
	102.5%MLSS[1]	0.14 (0.03)	0.82 (0.06)^{1}	0.82 (0.07)^{1}	0.82 (0.07)^{1}	0.81 (0.06)^{1,a,b}	0.81 (0.07)^{1}	0.81 (0.07)^{1}	0.81 (0.07)^{1}
$\dot{E}$[#*] (mL·kg^{-1}·min^{-1})	97.5%MLSS	9.4 (2.2)	42.8 (6.9)	42.3 (7.0)	41.9 (7.0)	41.4 (6.9)^{a,b}	41.7 (6.9)	42.5 (6.5)	42.0 (6.3)
	100%MLSS	9.2 (2.8)	46.0 (5.4)^{1}	46.3 (5.6)^{1}	45.3 (5.9)^{1}	44.5 (5.0)^{1,a,b}	45.2 (5.2)^{1}	45.0 (4.0)^{1}	44.8 (4.8)^{1}
	102.5%MLSS	8.3 (1.7)	48.7 (5.0)^{1,2}	48.9 (4.6)^{1,2}	49.1 (5.5)^{1,2}	48.2 (4.9)^{1,2,a,b}	48.0 (4.3)^{1,2}	48.4 (4.5)^{1,2}	48.1 (4.5)^{1,2}
SR[#*] (cycles·min^{-1})	97.5%MLSS	–	33.4 (3.3)	33.8 (3.4)	33.4 (3.3)	33.6 (3.4)	33.7 (3.2)^{a}	34.1 (3.6)^{a}	34.2 (3.5)^{a,c,d}
	100%MLSS	–	34.0 (3.3)	34.5 (3.0)^{1}	34.5 (3.1)^{1}	34.4 (3.2)^{1}	34.8 (3.6)^{1,a}	34.6 (3.8)^{1,a}	35.2 (3.8)^{1,a,c,d}
	102.5%MLSS	–	35.4 (3.3)	36.1 (3.3)^{1,2}	36.2 (3.1)^{1,2}	36.0 (3.3)^{1,2}	36.7 (2.8)^{1,2,a}	37.3 (3.3)^{1,2,a}	37.2 (3.3)^{1,2,a,c,d}
SL[#*] (m·cycle^{-1})	97.5%MLSS	–	2.18 (0.14)	2.16 (0.14)	2.18 (0.15)	2.17 (0.14)	2.16 (0.13)	2.14 (0.15)^{a}	2.13 (0.15)^{a,c,d}
	100%MLSS	–	2.20 (0.13)	2.16 (0.12)	2.17 (0.14)	2.17 (0.13)	2.15 (0.15)	2.16 (0.16)^{a}	2.13 (0.16)^{a,c,d}
	102.5%MLSS	–	2.16 (0.12)	2.12 (0.14)^{1,2}	2.11 (0.14)^{1,2}	2.12 (0.14)^{1,2}	2.08 (0.14)^{1,2}	2.05 (0.13)^{1,2,a}	2.06 (0.14)^{1,2,a,c,d}
SI[*] (m^{2}·s^{-1}·cycle^{-1})	97.5%MLSS	–	2.63 (0.20)	2.60 (0.20)	2.63 (0.23)	2.62 (0.18)	2.61 (0.19)	2.58 (0.21)^{a}	2.57 (0.20)^{a,c,d}
	100%MLSS	–	2.72 (0.19)	2.68 (0.21)	2.69 (0.22)	2.69 (0.19)	2.66 (0.21)	2.67 (0.21)^{a}	2.63 (0.21)^{a,c,d}
	102.5%MLSS	–	2.73 (0.18)	2.68 (0.22)	2.67 (0.24)	2.68 (0.22)	2.63 (0.25)	2.59 (0.21)^{a}	2.60 (0.22)^{a,c,d}

Intensity and/or time effect or interaction effect are indicated in each parameter.
[1,2] Values significantly different to 97.5 and 100%MLSS, respectively; [a,b,c,d] Values significantly different to the 4th min, 25, 33 and 50%, respectively ($p < 0.05$).
[#] Intensity effect.
[*] Time effect.
[†] Interaction effect.

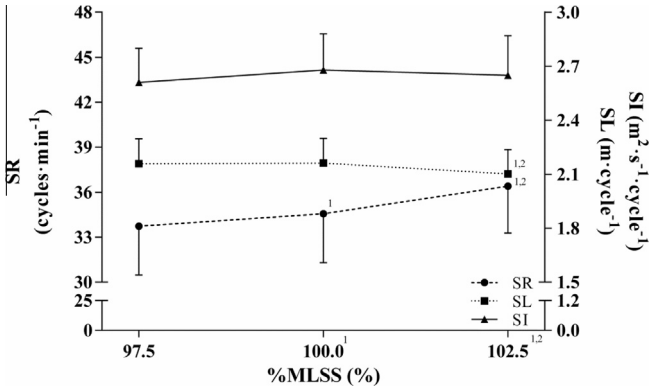

Fig. 2. Mean ± SEMI-SD of stroke rate (SR), stroke length (SL) and stroke index (SI) values at velocities corresponding to 97.5, 100 and 102.5% (slowest to highest velocity, respectively) of the maximal lactate steady state (MLSS) plotted as a function of velocity. [1,2] Values significantly different to 97.5 and 100% MLSS, respectively ($p < 0.05$).

evaluation of aerobic capacity in sports, including swimming (Beneke, 2003; Beneke & von Duvillard, 1996; Faude et al., 2009). The main findings of the present study were: (a) $\dot{V}O_2$, C and $\dot{E}$ were constant throughout the test time duration at each one of the three studied swimming intensities (97.5, 100 and 102.5%MLSS), while HR increased as a function of time for all the three v's studied; (b) at 97.5 and 100%MLSS, bioenergetical factors did not change as a function of time. However, SR increased and SL decreased as a function of time in the 30 min swims (biomechanical factors) and; (c) at 102.5%MLSS, although the $\dot{V}O_2$ and C were constant as a function of time, $\dot{V}E$ and HR increased as a function of time. With a constant $\dot{V}O_2$ and an increasing $\dot{V}E$, OUE decreased. The increased [La-] and $\dot{V}E$ are also likely responsible for eight out of ten of the subjects' not completing the 30 min swim at 102.5%MLSS due to locomotor and respiratory muscle fatigue (Harms, Wetter, St Croix, Pegelow, & Dempsey, 2000).

In the present study, gas exchange values ($\dot{V}O_2$ and $\dot{V}E$) were directly measured breath-by-breath as a function of time for intensities below, at, and above MLSS in swimming. However, previous researchers used measurements during the recovery period which were back extrapolated to assess $\dot{V}O_2$ and $\dot{V}E$ values during prolonged continuous swimming (Baron et al., 2005; Dekerle, Nesi et al., 2005). The $\dot{V}O_2$ values for 100%MLSS in women were lower in the present study (2.83 L.min^{-1}) compared to those previously reported for men during submaximal exercise that used the back extrapolation from recovery method (4.94 L.min^{-1}) (Dekerle, Nesi et al., 2005). The lower $\dot{V}O_2$ values in the present study could be explained by differences in the sex of the subjects studied. Women have been reported to have lower muscle mass, and differences in pulmonary structure (Hopkins & Harms, 2004; Sheel, Richards, Foster, & Guenette, 2004). This is supported by the well-established lower $\dot{V}O_2$ values for female athletes compared to their male counterparts (Rodriguez & Mader, 2011). In spite of the gender and method differences, the MLSS in the present study for women occurred at 85% (4% SD) of $\dot{V}O_{2max}$, which is similar to that reported in a previous study for men (86% $\dot{V}O_{2peak}$) (Dekerle, Nesi et al., 2005), the latter based on the measurements of recovery period.

The increase in $\dot{V}E$ values as function of time during the 30 min swim were stable at the lower v's. However, at the highest v studied (102.5%MLSS) $\dot{V}E$ increased as a function of time. The increased $\dot{V}E$ in this study is in accordance with a previous study (Baron et al., 2003), who reported similar values (~71.6 L.min^{-1}) for cycling at 100%MLSS. The time-dependent increase in $\dot{V}E$, with a constant $\dot{V}O_2$ and increased [La-] (hyperventilation), likely occurs as a respiratory compensation secondary to metabolic acidosis as seen in the present study, and likely due to the $\dot{E}$ of swimming at that velocity exceeding the aerobic supply capacity. The physiological system adjusts cardiopulmonary variables to match the oxygen delivery to the exercise intensity/v, i.e. the ratio between $\dot{V}O_2$ and $\dot{V}E$. The ratio of $\dot{V}O_2/\dot{V}E$ has been described as an index of ventilatory efficiency (OUE) (Baba et al., 1996), which has also been reported to vary among different athletes (Pelarigo et al., 2014), and was decreased in this study at the highest v, and as a function of time.

The observation that $\dot{V}O_2$ was unchanged while $\dot{V}E$ increased as a function of time demonstrates that the subjects were hyperventilation. The OUE in this study decreased as a function of time at all exercise intensities. At the lowest studied v, the mild decrease in OUE may be explained by a slight reduction in $\dot{V}O_2$ from the 4th min throughout the swim. The reduction in $\dot{V}O_2$ as a function of time may be explained by biomechanical adjustments to promote gross efficiency, with swimmers improving propelling efficiency and/or decreasing drag (Pelarigo et al., 2011; Toussaint & Hollander, 1994). At the fastest v (102.5%MLSS), OUE showed a greater, and significant decay as a function of time compared to the lower v's. The decrease of OUE as a function of time during the swim at 102.5MLSS is likely explained by the respiratory compensation for metabolic acidosis (increased [La-]), increasing $\dot{V}E$ when $\dot{V}O_2$ remained constant (Baba et al., 1996). Alternatively, there may be an increased pulmonary dead space due to the reduction in tidal volume, and resultant increase in breathing frequency to meet the increased $\dot{V}E$ needs as previously suggested (Baba et al., 1996). If the pulmonary dead space significantly increased during exercise, the $\dot{V}E$ would have to be increased to provide the same alveolar ventilation at a higher energy cost to the respiratory muscles (Harms et al., 2000). It is possible that the increased $\dot{V}E$ (hyperventilation) observed in this study was

respiratory compensation for metabolic acidosis due to the buildup of [La-] and also resulted in an increased pulmonary dead space. The increased [La-] and $\dot{V}E$ are also likely responsible for eight out of ten of the subjects' not completing the 30 min swim at 102.5%MLSS due to locomotor and respiratory muscle fatigue (Harms et al., 2000).

Supporting the role of respiratory compensation for metabolic acidosis at 102.5%MLSS is the reduced end-tidal P_aCO_2 (ETP_aCO_2) observed suggesting a decrease in arterial CO_2 (P_aCO_2). The lower P_aCO_2 is involved in the buffering system and control of metabolic acidosis. ETP_aCO_2 values at 102.5%MLSS was lower than at the two lower v's (97.5 and 100%MLSS) and than what is considered normal P_aCO_2 (40 mmHg). Furthermore, the ETP_aCO_2 values decreased as a function of time in the 102.5%MLSS swim from the start of the exercise (4th min) to its end for all swimming speeds. Thus, the reductions in ETP_aCO_2 as a function of time at all v's, and particularly for the 102.5%MLSS v confirm the respiratory compensation, and thus support its role in the reduced OUE.

Lower values of [La-] at 100%MLSS (1.89 $mmol.L^{-1}$) were observed in the females in the present study compared to swimming literature reported data for males (2.8–3.3 $mmol.L^{-1}$) (Dekerle, Nesi et al., 2005; Fernandes, Sousa, Machado, & Vilas-Boas, 2011; Pelarigo et al., 2011). Part of this difference in the [La-] values is due to the lower [La-] values commonly observed for women compared to men in middle-distance and endurance exercise (Crewther, Cronin, & Keogh, 2006; Greco, Pelarigo, Figueira, & Denadai, 2007; Holfelder, Brown, & Bubeck, 2013). The lower [La-]'s in women are likely explained by their lower body mass and lean muscle mass (Crewther et al., 2006; Holfelder et al., 2013). A further potential difference between men and women is the higher testosterone concentration in men (Deschenes & Kraemer, 2002), which could suggest a different metabolic balance between carbohydrates and fat throughout prolonged exercises (Greco et al., 2007; Tarnopolsky, Atkinson, Phillips, & MacDougall, 1995). Another potential factor for the low values of [La-] for women found in the present study may be due to their potentially greater adaptation of aerobic metabolism during exertion caused by their higher level of training than the men previously reported. Endurance athletes have been shown to have higher phenotypic expression of oxidative muscle fibers compared to sprint athletes (Tanaka & Swensen, 1998). This adaptation could lead to fibers that consume lactate (Gladden, 2008), supporting a physiological steady state at intensities near the $v\dot{V}O_{2max}$. These adaptations may explain the low final [La-] values observed in this study as the subjects were middle-distance and endurance swimmers.

The C (the ratio of $\dot{E}$ and v) has been suggested to be a major determinant of swimming performance (di Prampero, Pendergast, & Zamparo, 2011). $\dot{E}$ has to include both aerobic and anaerobic energy sources, especially at speeds near or greater than those than can be sustained using aerobic metabolism only (Fernandes et al., 2006). In the present study C was measured by direct methods during submaximal constant swimming for MLSS assessment and the anaerobic component from an energetic equivalent from blood [La-] (di Prampero et al., 2011). C increased around 5% as a function of v during the three swimming v's, as a linear function of v. Previous studies, however, reported that C increases with the v as a nonlinear function (di Prampero et al., 2011) or a cubic function (Rodriguez & Mader, 2011). This apparent discrepancy is likely explained by differences in the ranges of v's studied, more narrow in the present study and wider in the studies showing a nonlinear relationship for v. Previous studies used very low to maximal swimming v's (Rodriguez & Mader, 2011), contrasted to the v's used in this study (2.5% above and below 100%MLSS). Comparing v's at similar percentages of $v\dot{V}O_{2max}$ in front crawl swimming used in this study, C values of 37.9 $mL.m^{-1}$ (0.79 $kJ.m^{-1}$) and 41 $mL.m^{-1}$ (0.86 $kJ.m^{-1}$) for high level female swimmers at mean intensity values of 88% and 95% of maximum 400 m speed, respectively, have previously been reported (Chatard, Lavoie, & Lacour, 1991). The 400 m race in swimming has been highly associated with the minimum v that elicits $\dot{V}O_{2max}$ (Costill et al., 1985). Although the methodological approach (maximal 400 m *vs* progressive protocol) and the exercise mode (continuous *vs* intermittent) used in these studies were different (Chatard et al., 1991), the mean values of C were similar to those of the present study (0.78 and 0.81 $kJ.m^{-1}$ at 100 and 102.5MLSS, respectively).

HR presented similar adjustments as a function of time among the three swimming conditions in the present study, drifting up around 10 bpm from the 4th min to the end of the test. The increased HR observed in prolonged exercise, as in the present study, defined as "cardiovascular drift" (Fritzsche, Switzer, Hodgkinson, & Coyle, 1999), is likely explained by an increase in sympathetic nervous system activity and circulating norepinephrine concentrations, as well as other mechanisms to maintain cardiac output (Baron et al., 2008). The mean HR value at 100%MLSS was of 92% (SD 4%) of HR at $\dot{V}O_{2max}$ in the present study, a value which is similar to that previously reported (Dekerle, Nesi et al., 2005; Dekerle, Pelayo et al., 2005).

The v values at 100%MLSS for females reported in this study are similar to those reported for males in previous studies (Baron et al., 2005; Dekerle, Pelayo et al., 2005; Pelarigo et al., 2011). In addition the SR, SL and SI values reported in those same studies using men (Baron et al., 2005; Dekerle, Pelayo et al., 2005; Pelarigo et al., 2011) are similar to those reported in this study for women. These similarities of the male data from previous studies and the current study are most likely due to the high technical and training level of our swimmers. This assertion is strengthened by the v at 100%MLSS expressed as a percentage of $v\dot{V}O_{2max}$ observed in the present study (91.8 ± 4.6%) compared to previous reports in swimming (88.9 ± 3.3%) (Dekerle, Nesi et al., 2005), cycling (78.2 ± 4.9%), and running (75.9 ± 5.1%) (Figueira, Caputo, Pelarigo, & Denadai, 2008).

Swimming v is obtained by the product of SR and SL (Craig & Pendergast, 1979). It has also been shown that fatigue may interfere with the stroking parameters adopted by the swimmers to maintain a given v (Pelarigo et al., 2011). In the present study, swimmers did not sustain their SL at 102.5%MLSS compared to 97.5 and 100%MLSS, and thus they had to increased their SR to maintain their paced v. Indeed, the decline in SL as a function of v resulted in a decrease in SI, suggesting the importance of the ability to maintain biomechanical efficiency with exercise intensity. These findings are in agreement with previous studies, who reported decreases in SL and increases in SR in all-out distance trials (Craig & Pendergast, 1979) and with time at imposed paces (Dekerle, Nesi et al., 2005; Figueiredo et al., 2014; Pelarigo et al., 2011). In addition, SR values in

this study increased in the final periods of the 30 min swims compared to the beginning of the swims (4th min). Conversely, SL and SI were lower in the final periods of the swims compared with the beginning of the swims (4th min). A previous study (Dekerle, Nesi et al., 2005) reported that, at 100%MLSS, there was a slight decrease of SL (−3.3%) and increase of SR (3.6%) (non-significant in absolute values), from the beginning to the end of exercise, respectively. Similar trends in SL and SR were found in the present study during 100%MLSS exercise. Moreover, at 102.5%MLSS fatigue likely developed as a function of time and was associated with the SL decrease (−4%), and the SR increase (4.3%) from the beginning to the end of exercise. The biomechanical data show that for all three swimming v's, SR had to be increased to compensate for the reduced SL to keep v constant as set by the protocol. In spite of these stroke mechanics changes $\dot{E}$ and C were not affected. As $\dot{E}$ and C are determined by drag and net mechanical efficiency the absences of changes in $\dot{E}$ suggest either or both drag and net mechanical efficiency did not change.

5. Conclusions

Our results suggest that, at intensities up to the MLSS, bioenergetical and biomechanical factors are constant as a function of time in a 30 min swim. However, above the MLSS (102.5%MLSS) there was a decrease in ETP_aCO_2 which is a result of hyperventilation ($\dot{V}E/\dot{V}O_2$), most likely caused by respiratory compensation for metabolic acidosis (increased [La-]), thus decreasing OUE. As $\dot{V}O_2$ was constant over the 30 min swim at all v's, increased [La-] and SR most likely caused fatigue and, together, were associated with the inability of most swimmers to complete the 30 min swim at 102.5%MLSS.

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