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**original article**

# Cardiorespiratory response and energy expenditure during exercise at maximal lactate steady state

## *Resposta cardiorrespiratória e gasto energético em exercício na máxima fase estável de lactato*

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**Abstract** – There has been little research regarding cardiorespiratory responses during submaximal exercise at the maximal lactate steady state intensity ($MLSS_{int}$) until exhaustion. The objective of this study was to investigate the responses of oxygen consumption ($VO_2$), heart rate (HR) and oxygen pulse ($O_2$ pulse) during exercise to exhaustion at $MLSS_{int}$, and to compare energy expenditure (EE) estimated by $VO_2$ and HR. Twelve trained athletes followed an incremental protocol on a cycle ergometer to determine maximal and submaximal parameters of aerobic metabolism. On subsequent occasions they performed 2 to 4 30-minute tests with constant load to identify $MLSS_{int}$. Finally, they underwent a test to exhaustion at $MLSS_{int}$. Cardiorespiratory parameters were measured continuously during all tests. During the test to exhaustion, physiological responses were compared for six points in time calculated as percentages of the time to exhaustion (TTE). Mean TTE was 55.1±10.2 min. Oxygen pulse presented significant reduction over time, decreasing to a value 9% lower than baseline at the exhaustion point. This fact was the result of increases in HR over time that was disproportional to the increase in $VO_2$, resulting in significant differences between EE estimates. Therefore, there appears to be a gradual loss of cardiorespiratory efficiency during exercise to exhaustion at $MLSS_{int}$ that is shown by the reduction in $O_2$ pulse. The direct relationship between $VO_2$ and HR with workload presents variations over the course of exercise, leading to errors when EE is estimated using HR.

**Key words**: Cycling; Fatigue; Oxygen pulse; Submaximal exercise.

**Resumo** – *A resposta cardiorrespiratória durante exercícios submáximos, na intensidade da máxima fase estável de lactato ($MFEL_{int}$) até a exaustão, tem sido pouco investigada. O objetivo deste estudo foi investigar a resposta do consumo de oxigênio ($VO_2$), frequência cardíaca (FC) e pulso de oxigênio (pulso $O_2$) em exercício realizado na $MFEL_{int}$ até a exaustão, e comparar o gasto energético (GE) estimado pelo $VO_2$ e pela FC. Doze sujeitos treinados realizaram um protocolo incremental em cicloergômetro para determinar parâmetros máximos e submáximos do metabolismo aeróbio. Posteriormente, foram realizados 2 a 4 testes de 30 min com carga constante para identificar a $MFEL_{int}$. Finalmente, os sujeitos realizaram um teste até a exaustão na $MFEL_{int}$. Os parâmetros cardiorrespiratórios foram medidos continuamente durante todos os testes. No teste de exaustão, as respostas fisiológicas foram comparadas entre seis momentos relativos do tempo de exaustão (TTE). O TTE médio foi 55,1±10,2 min. O pulso de $O_2$ apresentou reduções significativas ao longo do tempo, atingindo, no momento da exaustão, um valor ~ 9% inferior comparado ao início do exercício. Este fato ocorreu pelo aumento da FC ao longo do tempo de forma desproporcional ao aumento do $VO_2$, resultando em diferenças significantes entre os GE estimados. Portanto, em exercício realizado na $MFEL_{int}$ até a exaustão, parece existir uma perda gradual da eficiência cardiorrespiratória, evidenciada pela redução do pulso $O_2$. Assim, a relação direta entre $VO_2$ e FC com a carga de trabalho é alterada ao longo do exercício, conduzindo a erros de estimativa do GE a partir dos valores da FC.*

**Palavras-chave**: *Ciclismo; Exercício submáximo; Fadiga; Pulso de oxigênio.*

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## INTRODUCTION

Understanding of cardiorespiratory and metabolic responses to exercise at different intensity domains is considered essential to prescription of long-duration aerobic exercise (> 30 minutes) and to designing experimental models[1]. To achieve this understanding, one of the most important physiological indices related to aerobic exercise performance is the intensity that provokes the maximal lactate steady state ($MLSS_{int}$), which is the greatest intensity of exercise at which maximum equilibrium is maintained between release of lactate into the bloodstream and its removal[2]. This intensity offers an interesting model for studying physiological responses during prolonged exercise, since it is located on the border between the domains of heavy and severe intensity exercise[2,3].

The behavior of oxygen consumption ($VO_2$) and heart rate (HR) during exercise at $MLSS_{int}$ is a subject that is receiving growing interest in many different studies in the scientific community[2-4]. However, there is still a gap in the literature, since there is insufficient knowledge about oxygen pulse ($O_2$ pulse, $mL.b^{-1}$) responses during exercise at $MLSS_{int}$. The $O_2$ pulse is defined as the quotient of $VO_2$ divided by HR and it is widely used for predicting systolic volume (SV) because the two variables are significantly correlated[5,6]. Monitoring this cardiovascular parameter during long-duration exercise is of interest because when exercise is at $MLSS_{int}$, $VO_2$ remains stable and HR increases progressively[2,3], reductions in $O_2$ pulse may indicate the occurrence of cardiovascular drift, which is characterized by an increase in HR in parallel with a reduction in SV, and may indirectly indicate compromised cardiorespiratory efficiency (i.e. a reduction in ventricular ejection fraction)[7,8].

Additionally, HR has been used to monitor exercise intensity[9] and, consequently, to estimate energy expenditure (EE), working from the assumption of a linear relationship between the values of HR and $VO_2$[10]. However, this relationship does not remain constant in long-duration exercise[11], meaning that EE estimates can be inaccurate if only HR is employed. The primary objective of this study was therefore to determine cardiorespiratory responses ($VO_2$, HR e $O_2$ pulse) during exercise at $MLSS_{int}$ until volitional exhaustion, using cycling as the exercise modality. The secondary objective was to compare EE estimated by means of equations based either on $VO_2$ or on HR.

## METHODOLOGICAL PROCEDURES

### Participants

Twelve trained male cyclists were enrolled on the study (29.2 ± 5.3 years; 176.5 ± 5.9 cm; 76.2 ± 6.8 kg). They had a minimum of 2 years' experience of cycling training and competition at the regional and state levels (mountain biking and road bike racing) and were training from 5 to 6 days per week with a weekly training distance of 320-360 km.

All of the procedures employed were approved in advance by the Human Research Ethics Committee at the Universidade Federal de Santa Catarina (protocol 056/2009). Additionally, all participants were informed of the risks and benefits involved, were familiarized with the experimental procedures and signed free and informed consent forms.

## Experimental procedure and materials used

Participants were instructed to arrive at the laboratory rested, and hydrated. They were also requested to avoid consuming caffeinated drinks for at least three hours beforehand and to avoid intense physical exercise for 48 hours prior to the tests. The entire experimental protocol was completed in under 3 weeks, with a minimum interval of 48 hours between each test. Additionally, each participant underwent all tests at the same time of day, with a maximum variation of 2 hours with relation to their first test time, in order to avoid interference from biological variations[12]. All tests were conducted in a climate-controlled environment with a mean temperature of 24.5±2.3°C and relative humidity of 55-60%.

Initially, the participants were subjected to a maximum incremental test in order to determine their anaerobic thresholds (AnT) and their maximum values for $VO_2$ ($VO_2$max), HR (HRmax), $O_2$ pulse ($O_2$ pulse-max) and maximum power (Pmax). The AnT was defined as the exercise intensity that corresponded to a fixed blood lactate concentration ([La]) of 3.5 $mmol.L^{-1}$ and this intensity was then used for the first test for determination of $MLSS_{int}$[13]. Blood samples (25 μL) for the [La] assays were taken from ear lobes using heparinized capillaries and stored in capped polyethylene microtubes (Eppendorf type), containing a solution at a known concentration (50 μL of sodium fluoride). Readings were taken using an electrochemical analyzer (*YSI 2700 STAT,* Yellow Springs, Ohio, USA), which was automatically calibrated after every 5 samples using a standard L-Lactate solution at 0.50g.L.

During the incremental test and the time to exhaustion test (TTE), $VO_2$ and ventilation (VE) were measured for every breath (Quark PFTergo, COSMED, Roma, Italy). The gas analyzer was calibrated immediately before each test, in accordance with the manufacturer's instructions. Heart rate was monitored constantly using a heart rate monitor connected to the gas analyzer. The $O_2$ pulse figures were obtained by dividing $VO_2$ by HR during the incremental protocol and the TTE. All tests were conducted on a cycle ergometer with an electromagnetic brake (Ergofit 167 Cycle, Pirmasens, Germany). Each athlete chose their preferred cadence, which varied from 75 to 90 rpm and was kept constant (± 5 rpm) throughout all procedures.

## Maximum Incremental Protocol

The maximum incremental test started at 105 W and increased by 35 W every 3 min until volitional exhaustion[14], during which time participants were verbally encouraged to achieve their maximum effort. The $VO_2$ and HR readings were reduced to means for 15 second intervals, and $VO_2$max and HRmax were defined as the highest reading. The test was defined as having achieved maximum when two or more of the following criteria were met: respiratory quotient > 1.10; $VO_2$ plateau; [La] > 8 $mmol.L^{-1}$ and/or 90% of estimated HRmax for age[15]. Blood samples were collected at the end of each stage of the protocol for measuring [La]. The following equation was used to calculate Pmax: Pmax(W) = load during last stage completed (W) + [t (s)/duration of stage (s) * load increment (W), where "t" is the time of the uncompleted stage[16].

## Protocol for determination of $MLSS_{int}$

After the incremental test, from two to four 30 min submaximal tests at constant load were conducted on different days in order to identify $MLSS_{int}$. Blood samples were taken for [La] assays at the 10th and 30th minutes of the tests. The $MLSS_{int}$ was defined as the highest intensity that could be maintained without causing an increase in [La] greater than or equal to 1 $mmol.L^{-1}$ during the last 20 minutes of the test[13,17]. The blood lactate concentration corresponding to $MLSS_{int}$ (the $MLSS_{[La]}$), was obtained by calculating the mean of the results for 10 and 30 minutes.

If the first test at constant load led to stabilization or reduction in [La] concentrations, then the next tests (on different days) were conducted with 5% increases in load per test until [La] no longer remained stable. In contrast, if [La] increased and/or the athlete could not complete the test due to exhaustion, subsequent tests were conducted with a 5% reduction in load. The athletes warmed up for 5 minutes at 50% of Pmax before each test to identify $MLSS_{int}$.

## Protocol for determination of $VO_2$, HR and $O_2$ pulse for TTE at $MLSS_{int}$

All participants undertook a TTE test at the $MLSS_{int}$ that had been determined previously. The criteria used to define exhaustion were either when the participant strayed from his preferred cadence (± 5 rpm) for the second time or when he stopped pedaling[18]. After the initial 30 minutes, each subject was given 100 mL of water every 10 minutes. The gas analyzer mask was removed for a maximum of 30 s in order to enable riders to drink, and then refitted. During the TTE test, $VO_2$, HR and $O_2$ pulse were measured continuously. However, since each participant had a different TTE (in minutes), variables were expressed and analyzed as percentages of TTE between 10 and 100% ($t_{10\%}$, $t_{20\%}$, $t_{40\%}$, $t_{60\%}$, $t_{80\%}$ and $t_{100\%}$). The figures taken were the means for each variable during the last minute that fell within each of the TTE percentages. All analyses were conducted by the same experienced investigator.

When calculating percentage increases in each of the physiological variables during the TTE test, the results for $t_{10\%}$ were used as the baseline, in order to exclude the initial cardiopulmonary adjustments that take place during the transition from rest to exercise.

## Determination of Energy Expenditure (EE)

Three different EE estimates were calculated from the results of the TTE test at $MLSS_{int}$. Two of these were calculated using the $VO_2$ results and one was calculated from HR, working from the assumption of a linear relationship between HR and $VO_2$. These EE estimates were calculated for each of the % TTE times described above.

The first, $EE_1$, was obtained from the raw figures taken directly from the gas analyzer software (Cosmed®, Quark PFTergo) during the TTE test, and was calculated by multiplying the absolute $VO_2$ by the caloric equivalent corresponding to the last minute for each %TTE time. This method ($EE_1$) was used as the standard reference method for estimating EE since it is based on the relationship between energy cost (kcal) and the ratio of the volume of $CO_2$ produced by the $VO_2$. In contrast, $EE_2$ was calculated using the metabolic equivalent (MET) provided by the same instrument (Quark PFTergo), using the following formula[19]: EE = MET * body mass/60. A $VO_2$ of 3.5 $mL.kg.^{-1}min^{-1}$ was defined as corresponding to 1 MET.

Finally, $EE_3$ was calculated as follows. First, EE during the maximum incremental protocol was calculated as the product of $VO_2$ and the caloric equivalent corresponding to the respiratory quotient at the end of each stage. Next, the linear relationship between HR and EE was used to generate individual regression equations to estimate EE from the HR figures from the TTE test at $MLSS_{int}$[20].

## Statistical analysis

Descriptive statistics are expressed as mean ± standard deviation. The Shapiro-Wilk test was used to test for normality of data. One-way ANOVA for repeated measures was used to analyze changes in each variable (HR, $VO_2$, $O_2$ pulse, and mean and total EE) at each TTE percentage time. Two-way ANOVA for repeated measures was used to compare the different EE estimation methods and changes in EE during the TTE test. Both analyses of variance were supplemented with the Bonferroni post hoc test. Pearson linear correlation analysis was used to analyze the relationship between $VO_2$ and HR during the TTE protocol.

In order to investigate total EE as estimated by each different method, 95% limits of agreement were calculated as described by Bland and Altman[21], to detect intraindividual variability. In these analyses, $EE_1$ was considered the reference method.

A significance level of 5% was adopted for all analyses. All statistical treatments were conducted using GraphPad Prism (v. 5.0 GraphPad Prism Software Inc, San Diego, CA).

# RESULTS

Table 1 lists the results for physiological variables during the incremental test, the protocol for determination of $MLSS_{int}$ and the TTE test.

**Table 1.** Physiological responses during the maximum incremental test and during the MLSSint protocol.

| Variables | Mean ± SD |
|---|---|
| $VO_2max$ (mL.kg$^{-1}$.min$^{-1}$) | 60.2 ± 7.8 |
| HRmax (bpm) | 193 ± 7 |
| $O_2$pulse-max (mL.b$^{-1}$) | 23.8 ± 2.9 |
| Pmax (W) | 341.1 ± 33.6 |
| $MLSS_{int}$ (W) | 253.3 ± 30.2 |
| $MLSS_{[La]}$ (mmol.L$^{-1}$) | 3.7 ± 0.7 |
| TTE (min) | 55.2 ± 10.1 |

Notes: $VO_2max$ – maximum oxygen uptake; HRmax – maximum heart rate; $O_2$pulse-max – maximum oxygen pulse; Pmax – maximum power; $MLSS_{int}$ – intensity corresponding to maximal lactate steady state, $MLSS_{[La]}$ – blood lactate concentration corresponding to $MLSS_{int}$, TTE – time to exhaustion.

Table 2 shows the behavior of HR, $VO_2$ and $O_2$ pulse during the TTE test used in this study. There was a progressive increase in HR reaching 13.3% at the end of the TTE test ($t_{100\%}$), whereas $VO_2$ stabilized by $t_{20\%}$. As a result, there were significant differences in $O_2$ pulse values from $t_{60\%}$ onwards (compared with $t_{10\%}$), reaching a mean reduction of 8.9% at the point of exhaustion. There were significant differences in HR, compared with the $t_{10\%}$ baseline, from $t_{20\%}$ onwards. In contrast, $VO_2$ only exhibited a significant increase over the $t_{10\%}$ value at $t_{80\%}$. Despite the fact that $VO_2$ and HR exhibited differing behavior, a high degree of correlation (r=0.87; p<0.05) was detected between the values of these two variables throughout the TTE test.

**Table 2.** Mean ± SD for behavior of physiological variables (HR, $VO_2$ and $O_2$ pulse) and percentage change (Δ) in each variable, at different percentages of TTE.

| | HR | | $VO_2$ | | $O_2$ pulse | |
|---|---|---|---|---|---|---|
| | bpm | Δ% | mL.kg$^{-1}$.min$^{-1}$ | Δ% | mL.b$^{-1}$ | Δ% |
| $t_{10\%}$ | 158 ± 9$^{*}$ | - | 46.4 ± 6.7$^{§}$ | - | 22.2 ± 2.2$^{//}$ | - |
| $t_{20\%}$ | 163 ± 8$^{*}$ | 3.0 ± 1.7 | 47.2 ± 7.2 | 1.7 ± 2.6 | 21.9 ± 2.4$^{//}$ | -1.1 ± 2.2 |
| $t_{40\%}$ | 167 ± 9$^{†}$ | 6.0 ± 2.3 | 47.9 ± 7.1 | 3.2 ± 4.4 | 21.5 ± 2.5$^{‡}$ | -3.3 ± 4.1 |
| $t_{60\%}$ | 172 ± 8$^{‡}$ | 8.8 ± 4.0 | 48.0 ± 7.8 | 3.3 ± 4.9 | 21.0 ± 2.4$^{‡}$ | -5.3 ± 4.7 |
| $t_{80\%}$ | 175 ± 9 | 10.8 ± 5.1 | 48.7 ± 8.0 | 4.9 ± 6.4 | 20.7 ± 2.6 | -6.7 ± 5.1 |
| $t_{100\%}$ | 179 ± 9 | 13.3 ± 5.9 | 48.0 ± 7.6 | 3.4 ± 6.1 | 20.2 ± 2.6 | -8.9 ± 6.0 |

Notes: HR – heart rate; $VO_2$ – oxygen consumption; $O_2$ pulse – oxygen pulse.
[*] $p < 0.05$ in relation to all other time percentages; [†] $p < 0.05$ in relation to $t_{80\%}$ and $t_{100\%}$; [‡] $p < 0.05$ in relation to $t_{100\%}$ only; [§] $p < 0.05$ in relation to $t_{80\%}$ only; [//] $p < 0.05$ in relation to $t_{60\%}$, $t_{80\%}$ and $t_{100\%}$.

Figure 1 illustrates the results for EE estimated by the three different methods. It will be observed that there was a significant increase in $EE_2$ values comparing $t_{10\%}$ with $t_{80\%}$, whereas $EE_3$ exhibited a significant increase, comparing baseline with the end of the test (15.4 vs. 19.4 kcal.min$^{-1}$, respectively). In contrast, $EE_1$ did not vary during the TTE test. Comparing the EE results obtained by each method it was observed that $EE_3$ was underestimated at $t_{10\%}$ and overestimated at $t_{100\%}$, when compared with methods based on $VO_2$. Notwithstanding, mean EE ($G_1$= 17.2 ± 1.7; $G_2$= 16.8 ± 1.6;

and $G_3$=17.4 ± 2.7 kcal.min$^{-1}$) and total EE ($G_1$= 958.5 ± 191.6; $G_2$= 940.3 ± 191.0; and $G_3$= 966.1 ± 207.6 kcal) estimated by each method did not differ significantly. Furthermore, the results of the analysis of agreement (95% limit of agreement) for total EE were 2.0 ± 1.7% ($EE_1$ and $EE_2$; Figure 2A) and 0.5 ± 25.6% ($EE_1$ and $EE_3$; Figure 2B).

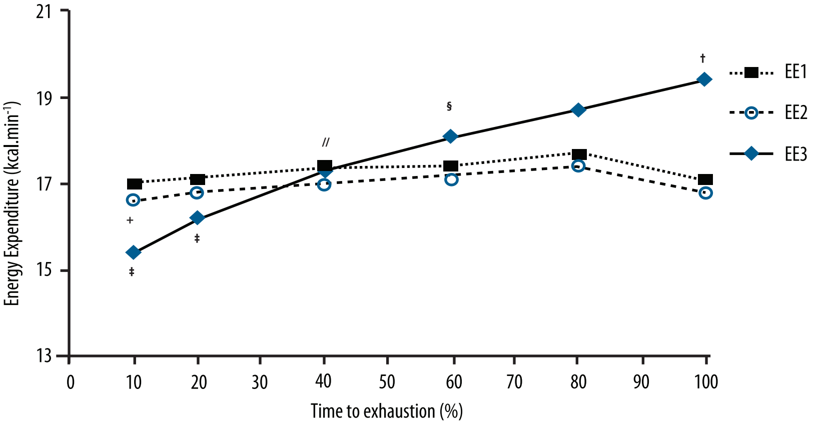

**Figure 1.** Comparison of energy expenditure (EE) estimated by three methods for each percentage of time to exhaustion.
Notes: EE1 – Energy expenditure according to analysis by Cosmed® software; EE2 – Energy expenditure according to metabolic equivalents (MET); EE3 – Energy expenditure by heart rate.
* $p < 0.05$ in relation to t10% for EE1 and EE2; † $p < 0.05$ in relation to t100% for EE1 and EE2; + $p < 0.05$ in relation to t80% for EE2; ‡ $p < 0.05$ in relation to t40%, t60%, t80% and t100% for EE3; // $p < 0.05$ in relation to t80% and t100% for EE3; § $p < 0.05$ in relation to t100% for EE3.

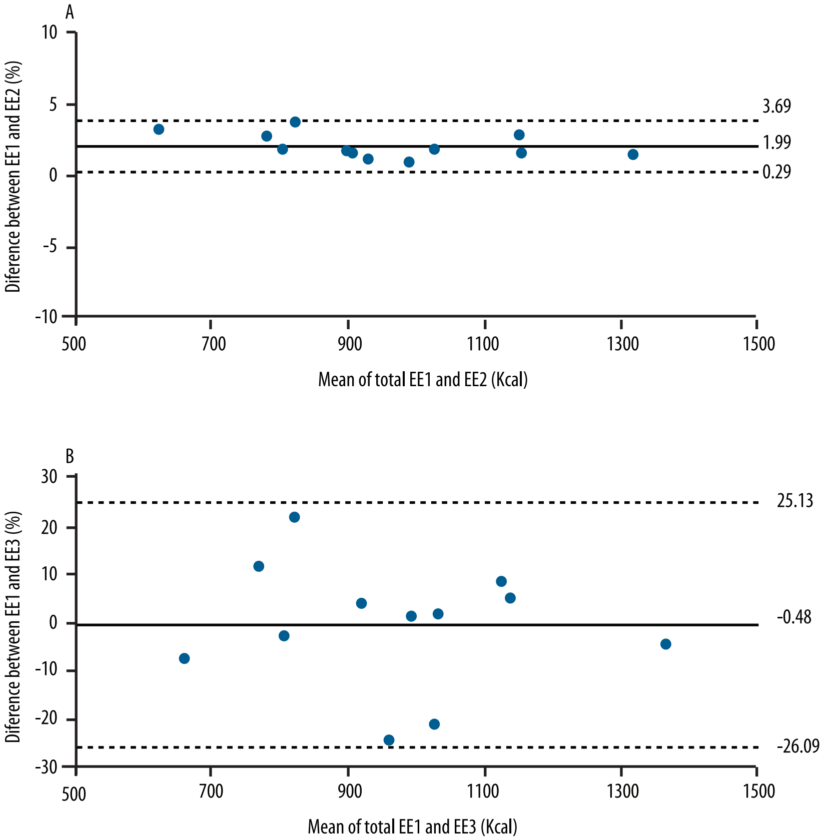

**Figure 2.** Bland-Altman analysis of agreement between EE1 and EE2 (A) and EE1 and EE3 (B).
Notes: EE1 – Energy expenditure according to analysis by Cosmed® software; EE2 – Energy expenditure according to metabolic equivalents (MET); EE3 – Energy expenditure by heart rate.

## DISCUSSION

The principal finding of this study was that there was a significant reduction in $O_2$ pulse accompanied by a progressive increase in HR (~ 13%) during exercise conducted at $MLSS_{int}$ until exhaustion. Additionally, increases in HR can cause overestimation of EE at certain points during long-duration exercise, particularly close to the point of exhaustion.

Some studies have found that $VO_2$ remains stable during exercise at $MLSS_{int}$[2,3]. However, in the present study a significant increase in $VO_2$ was observed during the protocol to exhaustion, comparing the $t_{10\%}$ baseline against $t_{80\%}$ (46.4 vs. 48.7 $mL.kg^{-1}.min^{-1}$, respectively). Barbosa et al.[22] and Lajoie et al.[4] have reported similar findings, observing increases in $VO_2$ in trained athletes, comparing the start and end of exercise after 30 and 60 minutes cycling at $MLSS_{int}$, respectively. However, in the present study, the $VO_2$ results for the point of exhaustion (t100%) were not different from baseline $VO_2$ from the start of exercise (t10%), suggesting that the difference observed may merely be the result of a statistical bias.

Studies of exercise undertaken at $MLSS_{int}$ have shown significant variations in other physiological variables, including HR, which increases continuously[2,3]. The HR response over the duration of the TTE protocol, which in our study led to a significant increase of 13.3%, has also been reported by other studies[2,4,8]. This increase may be explainable by greater activation of the sympathetic nervous system and higher concentrations of catecholamines in the bloodstream[3], and also by a progressive increase in motor unit recruitment via central command and/or muscle feedback[8]. Under conditions of hyperthermia and dehydration, HR will increase sufficiently to avert significant reductions in cardiac output. [23] This increase in HR during prolonged exercise, combined with a reduction in SV, has classically been described as cardiovascular drift[8,24,25].

The instability of the HR values observed in this study led to a progressive and significant reduction in $O_2$ pulse. Bhambhani et al.[6] have demonstrated the validity of using $O_2$ pulse to predict SV in trained male athletes during submaximal cycling exercise, finding a strong correlation ($r = 0.84$) between $O_2$ pulse and SV (obtained by dividing cardiac output by HR). It can be inferred from this that the drop in $O_2$ pulse is a reflection of the progressive reduction in SV, which characterizes cardiovascular drift and, consequently, indicates a reduction in ventricular ejection fraction.

Recent studies[11,25] conducted with trained cyclists during submaximal intensity exercise with indirect measures of cardiac output and SV may provide further support for the results of this study. Lafrenz et al.[25] observed significant cardiovascular drift, as reflected by changes in HR and SV, under differing experimental conditions (ambient temperature of 22°C vs. 35°C) during 45 minutes of exercise. However, the magnitude of cardiovascular drift was greater at the higher ambient temperature. Additionally, Ganio et al.[11] conducted experiments involving 120 minutes' exercise (ambient temperature of 30°C), with and without fluid intake (containing carbohydrate electrolytes), observing a significant increase in HR and a reduction in $O_2$ pulse during the first hour's exercise without fluid intake. During the second hour they observed a considerable reduction in SV, in addition to increases in the variations already observed. In contrast, when fluid intake was allowed, the magnitude of the *cardiovascular drift* was attenuated and $O_2$ pulse remained stable. Since the climatic and experimental conditions were different, comparisons of similar results from different studies should be made with caution. Notwithstanding, it is possible that the cause of the reduction in $O_2$ pulse observed in our study could be linked to fluid replacement with just ~300 mL of water not being sufficient to prevent the dehydration process. The recommendation for meeting fluid requirements is approximately 300-500 mL of water before exercise and 800-1600 $mL.h^{-1}$ of solutions containing 6% to 8% of carbohydrates during endurance activities lasting 1 to 3 hours[26].

Energy expenditure (EE) has been estimated for many different forms of exercise assuming that the relationship between $VO_2$, HR and aerobic metabolic demand is linear[10]. This linear relationship was indeed observed in the present study (r=0.87), despite the disproportional increase in HR in relation to $VO_2$ during the TTE protocol. The American College of Sports Medicine (ACSM) suggests using different equations to estimate $VO_2$ to thereby obtain EE during different forms of exercise. However, Londeree et al.[27] believe that these equations tend to underestimate $VO_2$ values for cycling, and as a consequence interfere with the EE estimates. To our knowledge, this is the first study to compare EE estimated by different methods, using just $VO_2$ and also EE estimated using HR values during exercise at $MLSS_{int}$ continued to volitional exhaustion.

Although in practice it is relatively uncommon that physical exercise is undertaken under constant load, the exercise model adopted here shows that mean and total EE did not exhibit any differences between estimates obtained directly from $VO_2$ and estimates obtained using HR. However, calculation of the limits of agreement showed that total $EE_3$ (estimated using HR) can vary by ± 25.6% compared with $EE_1$, suggesting a large degree of intraindividual variability. These findings show that total EE values based on HR could individually include considerable estimation error (± 25.6%) in relation to the reference method ($EE_1$). Similar results have been reported by Li et al.[28], who observed a wide range of interindividual (14.1 to 17.6%) and intraindividual (10.6 to 20.4%) variation when estimating energy expenditure from HR for a range of daily activities.

Additionally, $EE_3$ was underestimated at the start (t10%) and overestimated at the end (t100%) of the TTE test, compared with both $EE_1$ and $EE_2$ (Figure 1). Seen from this perspective, the progressive behavior of HR during exercise at $MLSS_{int}$ raises questions about the precision of using heart rate monitors as recommended for estimating the energy cost of activities included in physical exercise programs for athletes and active individuals[27]. This emphasizes the importance of undertaking further studies in the area of sports training to verify the validity of using $O_2$ pulse responses for assessing the ventricular ejection fraction and their relationship with fatigue during long-duration events.

Finally, certain methodological limitations must be acknowledged. First, it was not possible to measure SV during the TTE protocol adopted. Second, the level of dehydration of the athletes during the TTE test was not assessed, which to a certain point limits the extent to which physiological mechanisms can be used to explain the increase in HR and the consequent reduction in $O_2$ pulse.

## CONCLUSIONS

It can be concluded that HR was not stable over the course of exercise to exhaustion at $MLSS_{int}$, leading to a progressive reduction in $O_2$ pulse. The direct relationship between $VO_2$, HR and workload changes as exercise progresses, leading to EE estimation errors (± 25.6%) in relation to the reference method ($EE_1$) when calculations are based on HR. It is recommended that care is taken when using HR as a criterion for estimating EE during long-duration exercise under constant load.

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