

Research article

## Energy System Contributions during Incremental Exercise Test

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### Abstract

The main purpose of this study was to determine the relative contributions of the aerobic and glycolytic systems during an incremental exercise test (IET). Ten male recreational long-distance runners performed an IET consisting of three-minute incremental stages on a treadmill. The fractions of the contributions of the aerobic and glycolytic systems were calculated for each stage based on the oxygen uptake and the oxygen energy equivalents derived by blood lactate accumulation, respectively. Total metabolic demand ( $W_{TOTAL}$ ) was considered as the sum of these two energy systems. The aerobic ( $W_{AER}$ ) and glycolytic ( $W_{GLYCOL}$ ) system contributions were expressed as a percentage of the  $W_{TOTAL}$ . The results indicated that  $W_{AER}$  (86-95%) was significantly higher than  $W_{GLYCOL}$  (5-14%) throughout the IET ( $p < 0.05$ ). In addition, there was no evidence of the sudden increase in  $W_{GLYCOL}$  that has been previously reported to support to the “anaerobic threshold” concept. These data suggest that the aerobic metabolism is predominant throughout the IET and that energy system contributions undergo a slow transition from low to high intensity.

**Key words:** Oxygen uptake, blood lactate, aerobic threshold, anaerobic threshold, onset of blood lactate accumulation, energy metabolism contribution.

### Introduction

Traditionally, some of the most important physiological predictors of long-distance running performance have been measured using incremental exercise tests (IET) (Costill et al., 1973; Fairshier et al., 1983; Saltin and Astrand, 1967). The IET is a standard procedure for determining maximal and submaximal variables such as maximal oxygen uptake ( $VO_2max$ ) (Gordon et al., 2011) and metabolic thresholds (Kindermann et al., 1979), respectively. After the classic study of Hill and Lupton (1923), a large number of authors proposed that  $VO_2max$  could be used as a gold standard for maximal aerobic power evaluation (Gordon et al., 2011; Hottenrott et al., 2012; Saltin et al., 1967; Taylor et al., 1955). However, although  $VO_2max$  has been widely accepted as the upper limit of the body's aerobic functioning (Day et al., 2003), it has also been suggested that this variable is not sensitive enough to reflect changes in endurance capacity (Edwards et al., 2003) and may not be the best predictor of endurance performance in homogenous groups of athletes (Conley et al., 1980; Noakes et al., 1990) or in untrained individuals (Yoshida et al., 1987). Consequently, other submaximal physiological variables should also be con-

sidered during IET to better predict endurance performance (Bentley et al., 2007; Edwards et al., 2003; Hagberg et al., 1983).

It has been proposed that metabolic thresholds are useful physiological parameters to predict endurance performance because they reflect the adaptation from long-distance training (Edwards et al., 2003; Faude et al., 2009; Meyer et al., 2005). Historically, several methods have been proposed in the literature to determine these thresholds (see Svedahl and MacIntosh 2003 for review). Previous studies have demonstrated that both aerobic (Hagberg and Coyle 1983) and anaerobic (Svedenhag and Sjödin 1984) thresholds are important parameters for success in long-distance events. It is believed that high aerobic and anaerobic thresholds may reflect the ability of athletes to tolerate very high velocities during long periods of exercise (Edwards et al., 2003; Meyer et al., 2005).

In contrast, the terminologies utilized to discriminate metabolic thresholds may suggest an erroneous understanding about the profiles of metabolic pathways during an IET. As recently highlighted by Hopker et al. (2011), the term “anaerobic threshold” may not clearly indicate the moment at which the glycolytic system becomes the primary system resynthesizing ATP in exercised muscles. Although most sport scientists and coaches believe that metabolic pathways have a transitional nature, the profiles of aerobic and glycolytic metabolism during an IET have never been described in the literature. Understanding the relative aerobic and glycolytic contribution at each stage of an IET could provide a rational explanation for the human energy metabolism at a wide range of submaximal intensities. This information may lead to a more detailed understanding of the adaptations that result from the endurance training.

Therefore, the main purpose of the current study was to estimate the glycolytic and aerobic contributions to performance during IET. Because the anaerobic threshold is considered the highest intensity that can be maintained without considerable blood lactate accumulation (Faude et al. 2009), it was hypothesized that the glycolytic system would increase its contribution to total energy expenditure at work rates above the anaerobic threshold.

### Methods

#### Participants and procedures

Ten male recreational long-distance runners (age  $30 \pm 4$  years, body mass  $75.7 \pm 7.0$  kg, height  $1.78 \pm 0.06$  m, and

body fat  $15.2 \pm 3.8\%$ ) volunteered to participate in this study. All subjects were healthy non-smokers with no cardiovascular or neuromuscular diseases. They were not taking regular medication or nutritional supplementation. They had competed regularly in regional competitions and been training for the last two years without interruption. The runners were involved in local 10 km competitions and had personal records of  $40 \pm 5$  min. All runners performed only submaximal continuous aerobic training (50–70%  $\dot{V}O_{2\max}$ ) and had no previous high-intensity interval training or strength training experience. They were also familiar with exhaustive treadmill running.

The participants received verbal explanations about the possible benefits and risks associated with the study and signed a written informed consent before participating in the study. The IET was conducted during the preparatory training period under normal laboratory conditions (temperature  $22 \pm 1$  °C and relative humidity  $50 \pm 2\%$ ) at the same time of the day to avoid any possible diurnal variation effect on the performance. The subjects were instructed to refrain from exhaustive or unaccustomed exercises and the ingestion of caffeinated or alcoholic beverages for 24 hours before the tests. The procedures were in accordance with the Helsinki declaration of 1975, and the study was approved by the Ethics Committee for Human Studies of the School of Physical Education and Sport of the University of São Paulo.

### Incremental exercise test

Before starting the IET, participants were asked to rest quietly in a standing position for five minutes to determine the baseline  $\dot{V}O_2$  ( $\dot{V}O_{2\text{baseline}}$ ). After a three-minute warm up at  $6 \text{ km}\cdot\text{h}^{-1}$ , the speed was increased by  $1.2 \text{ km}\cdot\text{h}^{-1}$  every three minutes until volitional exhaustion (Heck et al., 1985). The treadmill (model TK35, CEFISE, Nova Odessa, Brazil) was set at a gradient of 1% throughout the IET to simulate outdoor running (Jones et al., 1996). A three-minute speed increment was chosen because it provides the most reliable and valid submaximal measurement of physiological variables (i.e., blood lactate and oxygen uptake) (Bentley et al., 2007). Subjects received strong verbal encouragement to continue as long as possible.

Each stage was separated by a 10-second rest period, during which capillary blood samples were obtained from the earlobe ( $25 \mu\text{L}$ ) and analyzed for blood lactate concentrations (YSI 1500 Sport, Yellow Springs Instruments, Yellow Springs, OH). Oxygen uptake ( $\dot{V}O_2$ ) was measured breath-by-breath throughout the tests using a gas analyzer (Metyzer 3b, Cortex, Leipzig, Germany). Calibration of the device was performed according to manufacturer specifications using ambient air, a gas of known composition containing 20.9%  $\text{O}_2$  and 5%  $\text{CO}_2$ , and a 3 L syringe (Model 5530 Series, Hans Rudolph, Kansas City, USA). Heart rate was measured during the test with a heart rate monitor (S810, Polar Electro Oy, Kempele, Finland) that was coupled to the gas analyzer.

The  $\dot{V}O_{2\max}$  was determined when two or more of the following criteria were met: an increase in  $\dot{V}O_2$  of less than  $2.1 \text{ ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  on two consecutive final stages, a respiratory exchange ratio greater than 1.1, a blood lactate

concentration higher than  $8.0 \text{ mmol}\cdot\text{l}^{-1}$ , and a heart rate  $\pm 10$  bpm of the maximal age-predicted heart rate (Howley et al., 1995). The speed corresponding to  $\dot{V}O_{2\max}$  ( $V_{\max}$ ) was established as the minimal speed that elicited  $\dot{V}O_{2\max}$  (Bertuzzi et al., 2012). If a runner achieved  $\dot{V}O_{2\max}$  during a stage that could not be maintained for one minute, the speed in the previous stage was defined as  $V_{\max}$ . The aerobic threshold was considered as the speed corresponding to blood lactate accumulation of  $2 \text{ mmol}\cdot\text{l}^{-1}$  as suggested by Kindermann et al. (1979), while the anaerobic threshold was the speed corresponding to  $3.5 \text{ mmol}\cdot\text{l}^{-1}$  of blood lactate accumulation as proposed by Heck et al. (1985). The highest speed achieved during the last completed stage was considered to be the peak treadmill velocity (Noakes et al., 1990). The maximal heart rate ( $\text{HR}_{\max}$ ) was defined as the highest value obtained at the end of the test.

### Calculation of the energy system contributions

Energy system contributions during each stage of the IET were determined as previously described (Bertuzzi et al., 2007; Bertuzzi et al., 2010). Briefly, the net aerobic energy system was estimated by subtracting  $\dot{V}O_{2\text{baseline}}$  from the  $\dot{V}O_2$  exercise area integrated over time by the trapezoidal method. To estimate the glycolytic system contribution, a value of  $1 \text{ mmol}\cdot\text{l}^{-1}$   $[\text{La}]_{\text{net}}$  was considered to be equivalent to  $3 \text{ ml O}_2\cdot\text{kg}^{-1}$  body mass (di Prampero and Ferretti 1999). As the ATP-PCr system is predominantly used to resynthesize of ATP during high-intensity (3–5 times the power output that elicits  $\dot{V}O_{2\max}$ ), short-duration exercises ( $\sim 10$  seconds) (Gastin 2001), the contribution of alactic metabolism was not considered. The total metabolic demand ( $W_{\text{TOTAL}}$ ) was the sum of both energy metabolism contributions. The aerobic ( $W_{\text{AER}}$ ) and glycolytic ( $W_{\text{GLYCOL}}$ ) energy system contributions were also expressed as a percentage of the  $W_{\text{TOTAL}}$ . A caloric equivalent of  $20.9 \text{ kJ}\cdot\text{l O}_2^{-1}$  was used for the energy systems calculations.

### Statistical analysis

The distribution of the data was analyzed by the Shapiro-Wilk test, and the results showed a normal Gaussian distribution. The data are reported as the mean and standard deviation (SD). A paired *t*-test was used to compare the  $W_{\text{AER}}$  and  $W_{\text{GLYCOL}}$  contributions estimated during the IET. Repeated measure analyses of variance with Bonferroni correction for multiple comparisons were used to compare the  $W_{\text{AER}}$  or  $W_{\text{GLYCOL}}$  contribution among the stages of the IET. The level of significance was set at  $p < 0.05$ .

### Results

Table 1 shows the physiological and performance parameters measured during the IET. Table 2 shows the cardiorespiratory variables and blood lactate accumulation during the IET until  $18 \text{ km}\cdot\text{h}^{-1}$ , which was the last completed stage. Lower values of minute ventilation ( $F_{(2,10)} = 136.9$ ,  $p < 0.05$ ), blood lactate accumulation ( $F_{(2,10)} = 19.3$ ,  $p < 0.05$ ), and heart rate ( $F_{(2,10)} = 320.1$ ,  $p < 0.05$ ) measured during early stages ( $6\text{--}9.6 \text{ km}\cdot\text{h}^{-1}$ ) were

**Table 2.** Cardiorespiratory variables and blood lactate accumulation for each stage of the incremental exercise test. Data are means ( $\pm$ SD,  $n=10$ ).

Speed (km·h <sup>-1</sup> )	6.0	7.2	8.4	9.6	10.8	12.0	13.2	14.4	15.6	16.8	18.0
<b>V<sub>E</sub></b> (l·min <sup>-1</sup> )	40.7 (6.4)	52.8 <sup>ab</sup> (7.9)	58.9 (7.1)	65.7 <sup>b</sup> (9.7)	74.0 (10.8)	81.3 (8.6)	91.5 (9.9)	118.6 (14.5)	122.3 (13.4)	122.3 (10.0)	143.0 (12.0)
<b>RER</b>	.84 (.05)	.92 (.03)	.95 (.04)	.96 (.04)	1.00 (.06)	1.01 (.05)	1.05 (.07)	1.10 (.08)	1.14 (.10)	1.14 (.07)	1.15 (.09)
<b>[La]</b> (mmol·l <sup>-1</sup> )	1.3 <sup>b</sup> (.5)	1.3 <sup>b</sup> (.4)	1.3 <sup>b</sup> (.3)	1.8 <sup>b</sup> (.7)	1.8 <sup>b</sup> (.6)	2.2 <sup>b</sup> (.8)	2.6 <sup>b</sup> (1.0)	3.55 <sup>b</sup> (1.1)	4.7 (1.2)	5.7 (1.6)	8.3 (1.8)
<b>HR</b> (bpm)	110 <sup>cd</sup> (15)	127 <sup>d</sup> (15)	135 (16)	144 (18)	152 (18)	160 (17)	167 (15)	179 (14)	180 (13)	181 (11)	183 (12)

V<sub>E</sub>: minute ventilation, RER: respiratory exchange ratio, [La]: blood lactate accumulation, HR: heart rate

<sup>a</sup> Significantly different from 12.0 km·h<sup>-1</sup>, <sup>b</sup> Significantly different from 18.0 km·h<sup>-1</sup>, <sup>c</sup> Significantly different from 8.4 km·h<sup>-1</sup>, <sup>d</sup> Significantly different from 9.6 km·h<sup>-1</sup>,  $p < 0.05$

observed compared with those during more intense stages. No significant difference was found in the respiratory exchange ratio throughout the IET after Bonferroni correction for multiple comparisons ( $F_{(2,10)} = 27.9$ ,  $p > 0.05$ ).

**Table 1.** Physiological and performance parameters measured during the incremental exercise test. Data are means ( $\pm$ SD,  $n=10$ ).

<b>VO<sub>2</sub>max</b> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	53.4 (2.0)
<b>HRmax</b> (bpm)	191 (3)
<b>PTV</b> (km·h <sup>-1</sup> )	18.0 (.0)
<b>Vmax</b> (km·h <sup>-1</sup> )	17.5 (1.0)
<b>Aerobic threshold</b> (km·h <sup>-1</sup> )	12.0 (1.0)
<b>Aerobic threshold</b> (%PTV)	76.0 (2.0)
<b>Anaerobic threshold</b> (km·h <sup>-1</sup> )	14.4 (1.0)
<b>Anaerobic threshold</b> (%PTV)	86.0 (1.0)

VO<sub>2</sub>max: maximal oxygen uptake; HRmax: maximal heart rate; PTV: peak treadmill velocity, Vmax: minimal velocity corresponding to VO<sub>2</sub>max; Aerobic threshold was determined at the velocity correspondent to 2 mmol·l<sup>-1</sup> of blood lactate accumulation, and anaerobic threshold at the velocity correspondent to 3.5 mmol·l<sup>-1</sup> of blood lactate accumulation.

Table 3 shows the absolute energy system contributions estimated for each stage. W<sub>AER</sub> was significantly higher when compared with W<sub>GLYCOL</sub> throughout the IET ( $p < 0.05$ ). The W<sub>AER</sub> increased continuously up to 183.9  $\pm$  23.5 kJ, while W<sub>GLYCOL</sub> increased continuously up to 30.6  $\pm$  3.0 kJ. However, this increase was not statistically significant in both W<sub>AER</sub> ( $F_{(2,10)} = 319.6$ ) and W<sub>GLYCOL</sub> ( $F_{(2,10)} = 23.6$ ) after Bonferroni correction for multiple comparisons ( $p > 0.05$ ).

Figure 1 shows the energy system interaction expressed as percentage of W<sub>TOTAL</sub>. The relative W<sub>AER</sub> contribution was statistically higher than W<sub>GLYCOL</sub> during all the stages of the IET ( $p < 0.05$ ). The aerobic contribution decreased continuously from 95.7  $\pm$  1.5% at 6 km·h<sup>-1</sup> to 86.1  $\pm$  4.7% at 18 km·h<sup>-1</sup>, while the W<sub>GLYCOL</sub> increased

continuously from 5.3  $\pm$  1.5% to 13.9  $\pm$  4.7%. However, this increase in relative W<sub>GLYCOL</sub> ( $F_{(2,10)} = 11.7$ ) and reduction in relative W<sub>AER</sub> ( $F_{(2,10)} = 11.5$ ) were not statistically significant throughout the IET after Bonferroni correction for multiple comparisons ( $p > 0.05$ ).

## Discussion

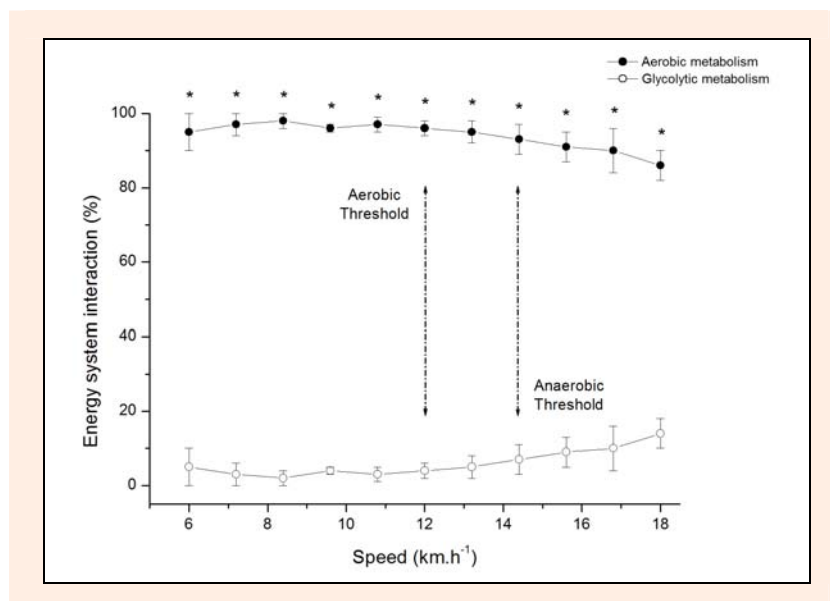
It has been recently emphasized that the terminologies utilized to describe metabolic threshold concepts may introduce an erroneous understanding about metabolic pathway profiles during the IET, especially because the term “anaerobic threshold” may not clearly indicate the predominance of the glycolytic energy system in ATP resynthesis (Hopker et al., 2011). It is known that both pyruvate and lactate are the end products of the anaerobic glycolysis. Lactate formation via enzyme lactate dehydrogenase is caused by a local mass action of high pyruvate and NADH concentrations. This suggests that high lactate concentrations detected in the blood could indicate the predominance of the glycolytic pathway. However, comparisons between W<sub>AER</sub> and W<sub>GLYCOL</sub> during graded incremental exercise tests had not been reported in the literature yet. The results of the present study revealed that the relative W<sub>AER</sub> progressively decreased by only ~9% from the first to the last stage, suggesting that aerobic metabolism is predominant throughout IET. In addition, no evidence was observed of a sudden increase in glycolytic contribution during the IET, which is the basis of the “anaerobic threshold” concept.

Aerobic threshold has been considered to be the upper limit of a nearly exclusive aerobic metabolism based mainly on the blood lactate concentration (Kindermann et al., 1979) and gas exchange (Meyer et al., 2005) responses. In the present study, we examined the W<sub>AER</sub> and W<sub>GLYCOL</sub> using the oxygen uptake area and oxygen

**Table 3.** Absolute energy system contributions in each stage of the incremental exercise test. Data are means ( $\pm$ SD,  $n=10$ ).

Speed (km·h <sup>-1</sup> )	6.0	7.2	8.4	9.6	10.8	12.0	13.2	14.4	15.6	16.8	18.0
<b>Aerobic</b> (kJ)	47.2 * (7.6)	63.0 * (7.7)	94.4 * (13.0)	110.2 * (15.0)	123.7 * (17.3)	134.0 * (16.4)	147.2 * (17.1)	160.2 * (15.5)	168.8 * (18.4)	177.0 * (22.1)	183.9 * (23.5)
<b>Glycolytic</b> (kJ)	2.1 (1.0)	2.0 (.9)	2.2 (1.1)	4.3 (1.0)	4.4 (1.6)	6.0 (1.2)	8.1 (2.0)	12 (2.3)	17.9 (2.5)	20.3 (3.1)	30.6 (3.0)
<b>W<sub>TOTAL</sub></b> (kJ)	49.3 (7.4)	65.1 (7.7)	96.6 (12.3)	114.4 (15.0)	128.1 (17.6)	140.0 (17.4)	155.3 (18.7)	172.1 (19.7)	186.7 (25.0)	197.3 (31.1)	214.6 (25.8)

W<sub>TOTAL</sub>: total metabolic demand. \* Different from glycolytic contribution ( $p < 0.05$ )



**Figure 1.** Energy system contributions during incremental exercise test expressed as a percentage of the total metabolic demand. Aerobic threshold was determined at the speed correspondent to 2 mmol·l<sup>-1</sup> of blood lactate accumulation. Anaerobic threshold was determined at the speed correspondent to 3.5 mmol·l<sup>-1</sup> of blood lactate accumulation. Values are given as mean ± SD (n = 10). \* Different from glycolytic contribution (p < 0.05).

equivalent for blood lactate accumulation, respectively. This approach allowed a direct comparison between the metabolism pathway contributions during the IET. Our results support previous findings in which the highest aerobic contribution was found at the speed corresponding to the aerobic threshold (Faude et al., 2009). In addition, although no significant difference was found between the contributions of both energy pathways throughout the IET, the speed corresponding to the aerobic threshold was the last stage before the initiation of a slow consistent decrease in the relative  $W_{AER}$  (Figure 1). Despite the fact that substrate oxidation was not determined in the present study, this may be related to the fat oxidation that occurs in exercises with low intensities. Previous findings have suggested a non-significant difference between the intensity corresponding to the aerobic threshold and the intensity in which the highest rate of fat oxidation is elicited in healthy men (Achten and Jeukendrup 2004). It has been proposed that the first rise in lactate concentration occurs at the same intensity in which maximal fat oxidation rate is elicited. This occurs because the increased glycolytic flux appears to inhibit the long-chain free fatty acid entry in the mitochondria (Achten and Jeukendrup 2004). Thus, the highest relative  $W_{AER}$  observed at the aerobic threshold may reflect the oxygen required for the fat oxidation by slow twitch fibers in moderately trained long-distance runners.

Notably, this highest relative  $W_{AER}$  observed at aerobic threshold can reflect the optimal stimulus to produce the adaptations related to endurance performance. For instance, Seiler and Kjerland (2006) analyzed the intensities chosen by highly trained cross-country skiers ( $VO_{2max}$  ~73 ml·kg<sup>-1</sup>·min<sup>-1</sup>) during a pre-competition preparation period using a three intensity-zone model. It was observed that the athletes performed 75%, 5-10%, and 15-20% of the total training duration at intensities corresponding to the aerobic threshold, between the aero-

bic and anaerobic thresholds, and above the anaerobic threshold, respectively. These findings demonstrated that long-distance athletes preferentially train at intensities close to the aerobic threshold. According to Seiler and Kjerland (2006), the training time spent at intensities slightly below the aerobic threshold is an important source of stress, which generates a high-oxidative flux in the working muscles during multiple daily sessions.

In contrast to our hypothesis, there was no significant rise in  $W_{GLYCOL}$  at intensities between the anaerobic threshold and  $VO_{2max}$ . Some studies have emphasized the importance of the utilized terminologies to discriminate between metabolic thresholds (Myers and Ashley 1997). Specifically, it was suggested that the term “anaerobic threshold” may not clearly indicate that the  $W_{GLYCOL}$  becomes predominantly utilized for ATP resynthesis in exercising muscles at intensities above the anaerobic threshold (Hopker et al., 2011). Similar to the aerobic threshold concept, the anaerobic threshold concept was proposed based only on the blood lactate responses during the IET (Meyer et al., 2005). To date, comparisons between  $W_{AER}$  and  $W_{GLYCOL}$  at or above the threshold have not been reported. Our data have revealed that  $W_{AER}$  and  $W_{GLYCOL}$  at the anaerobic threshold corresponded to ~93% and ~7% of total metabolic demand, respectively. This means that despite increased  $W_{GLYCOL}$  at intensities at or above the anaerobic threshold, the  $W_{AER}$  was predominant during all stages of the IET. Therefore, the ability of the anaerobic threshold to predict endurance performance may not be directly (i.e., ATP resynthesis) or indirectly (i.e., metabolite accumulation) linked to glycolytic metabolism.

Data from the current study provide a rational basis for the interactions between energy metabolism systems at a wide range of submaximal intensities. It was found that  $W_{AER}$  contributed ~95% and ~86% to the total energy expenditure at the start and end of IET, respec-

tively. This corroborates previous findings suggesting that  $W_{\text{GLYCOL}}$  has a significant contribution only at intensities above  $\text{VO}_{2\text{max}}$ . Using the maximal accumulated oxygen deficit method, Mezzani et al. (2008) reported that in patients with left ventricular dysfunction and healthy subjects,  $W_{\text{GLYCOL}}$  contributed ~35% and 27% of the total energy expenditure at intensities corresponding to 133% and 121% of  $\text{VO}_{2\text{max}}$ , respectively. Utilizing a similar method, Spencer and Gastin (2001) showed that in highly trained athletes, the relative contributions of the  $W_{\text{GLYCOL}}$  at running events were ~71, ~57, ~44, and ~16% at intensities corresponding to ~200, ~150, ~113, and ~103% of  $\text{VO}_{2\text{max}}$ , respectively. Therefore, our results complement a growing body of work demonstrating that  $W_{\text{GLYCOL}}$  is responsible for a negligible contribution to the total energy expenditure at intensities at and below  $\text{VO}_{2\text{max}}$ .

Some potential limitations should be considered when interpreting the current findings. Previous studies have reported restrictions related to the usage of  $\text{VO}_2$  and  $[\text{La}^-]$  to represent aerobic and glycolytic metabolism, respectively (Gastin 1994; Gladden 2004; Green and Dawson 1993). It has been suggested the determination of the energy pathway contributions of active muscles through the assessment of whole-body physiological variables can be inaccurate. For example, the appearance of slow component of  $\text{VO}_2$  would overestimate the  $W_{\text{AER}}$  contribution (Mezzani et al., 2008). However, it is possible that this had no influence on our calculations because the three-minute stage duration was too short to allow the slow component to appear. Regarding the determination of the glycolytic metabolism contribution, it is relevant to notice that lactate has been considered an important carbohydrate fuel source during exercise (Gladden 2008, Gladden 2004). Muscle lactate oxidation in skeletal muscle mitochondria involves a sequential mechanism in which protons and lactate molecules bind to the monocarboxylate transporter 1 (MCT1). Upon the start of the moderate intensity exercise, lactate and pyruvate concentrations increase at low levels without changing lactate/pyruvate ratio until a threshold at which lactate abruptly increases without pyruvate changes (Wasserman et al., 1985). Because of its higher concentration compared to pyruvate, muscle lactate would be transported across the inner mitochondrial membrane by MCT1 (Gladden 2008). Consequently, muscle lactate may be removed by a cell-to-cell lactate shuttle and used as an energy fuel by oxidative muscle fibers before it escapes into circulation. As a result, the estimated value of the glycolytic metabolism contribution based on blood lactate accumulation could be underestimated, especially at intensities above the anaerobic threshold. However, it has been showed that the lactate/pyruvate ratio increases in a similar manner as the  $\text{VO}_2$  responses during the IET (Wasserman et al., 1985). Consequently, the  $3 \text{ ml O}_2 \cdot \text{kg}^{-1} \cdot \text{mmol}^{-1} \text{ lactate} \cdot \text{L}^{-1}$  equivalent could be used as an empirical method that allows us to estimate the glycolytic energy release during dynamic exercise.

Alternatively, intramuscular metabolites could be used to quantify the glycolytic contribution during the IET. However, the muscle tissue used for these measurements is usually obtained from small muscle mass. Con-

sequently, it would be necessary to determine the muscle mass that is involved in a specific exercise, which could result in an inaccurate representation of glycolytic metabolism activation (Gastin 1994). In addition, the muscle biopsy technique requires specialized personnel, and athletes are often uncomfortable participating in studies that use this procedure, especially when many biopsies are required.

## Conclusion

Data from the present study demonstrate that the highest  $W_{\text{AER}}$  as well as the beginning of the aerobic/glycolytic transition occurs at a speed close to the aerobic threshold. In addition,  $W_{\text{AER}}$  and  $W_{\text{GLYCOL}}$  demonstrate a very slow progressive transition pattern throughout the IET, without the sudden increase in  $W_{\text{GLYCOL}}$  espoused by the “anaerobic threshold” concept.

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### Key points

- The aerobic metabolism contribution is the predominant throughout the maximal incremental test.
- The speed corresponding to the aerobic threshold can be considered the point in which aerobic metabolism reaches its maximal contribution.
- Glycolytic metabolism did not contribute largely to the energy expenditure at intensities above the anaerobic threshold.

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