

Research article

## Effect of Endurance Training on The Lactate and Glucose Minimum Intensities

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### Abstract

Due to the controversy about the sensitive of lactate minimum intensity (LMI) to training and the need to develop other tool for aerobic fitness evaluation, the purpose of this study was to analyze the sensitivity of glucose minimum intensity (GMI) and LMI to endurance training. Eight trained male cyclists ( $21.4 \pm 1.9$  years,  $67.6 \pm 7.5$  kg and  $1.72 \pm 0.10$  m) were evaluated twice, before and after 12 weeks of training. GMI and LMI were calculated, respectively, by the lowest blood glucose and lactate values attained during an incremental test performed after a hyperlactemia induction, and  $VO_{2max}$  was determined during standard incremental effort. The training was prescribed in three different zones and controlled by heart rate (HR). The training distribution was equivalent to 59.7%, 25.0% and 15.3% below, at and above anaerobic threshold HR respectively. The anaerobic threshold evaluated by GMI and LMI improvement  $9.89 \pm 4.35\%$  and  $10.28 \pm 9.89$  respectively, after training, but the  $VO_{2max}$   $2.52 \pm 1.81\%$ . No differences were found between GMI and LMI in pre ( $218.2 \pm 22.1$  vs  $215.0 \pm 18.6$  W) and post ( $240.6 \pm 22.9$  vs  $237.5 \pm 18.8$  W) training situations. LMI and GMI were sensitive to 12-week aerobic training in cyclist; thus, both protocols can be used to assess aerobic adaptation, athletes diagnostic and prescribe training.

**Key words:** Anaerobic threshold, endurance capacity, cyclists.

### Introduction

Anaerobic threshold (LTAN) or the exercise intensity above which blood lactate increases abruptly during graded effort has been extensively used in science, sport and clinical fields as an important index of aerobic fitness (McGehee et al., 2005; Simoes et al., 2009). Endurance performance prediction (Simoes et al., 1999), prescription of optimal training intensities (Sotero et al., 2009) and assessment of training induced adaptations (Edge et al., 2005; Junior et al., 1998) are important applications of LTAN. As a composite of aerobic fitness, oxygen uptake, the movement economy and the LTAN is classified a better indicators of aerobic fitness than only  $VO_{2max}$  (Dotan et al., 2011).

In recent years, great attention has been devoted to the lactate minimum test (LMT) for LTAN determination (Simoes et al., 2009; Sotero et al., 2009). This test consists of an incremental exercise session started eight minutes after maximal efforts. Since blood lactate show an U-shaped response during the graded phase, its lowest level theoretically represents the maximal lactate steady state (MLSS),

i.e., the equilibrium point (Tegtbur et al., 1993). Recently, Dotan et al. (Dotan et al., 2011) showed that the LMT present superior reliability than a typical progressive lactate-response test.

While some studies presented validity of lactate minimum intensity (LMI) for MLSS prediction (Gondim et al., 2007; MacIntosh et al., 2002; Sotero Rda et al., 2009), others show that LMT is affected by different protocol manipulations (Carter et al., 1999b; Pardonno et al., 2008; Ribeiro et al., 2009) and, Carter et al. (Carter et al., 1999a) questioned the sensitivity of LMT to changes in aerobic fitness, as a result of six weeks of endurance training in healthy students. However, methodological bias could have influenced these results (Carter et al., 1999a), since that LMT is protocol dependent (Johnson and Sharpe, 2011). Despite of great attention for this protocol, others (Junior et al., 2001; Simoes et al., 2003; Simoes et al., 1999) focused on simpler alternative methods for LTAN prediction.

Simões et al. (Simoes et al., 1999) reported the lowest blood glucose (BGI) as a good predictor of individual LTAN and LMI during track tests in endurance runners, although the authors point out that this new methodology must be extensively examined. Rocha et al. (Rocha et al., 2010) shown significant improvement of individual glucose threshold after military training. However there is no available literature concerning the sensitivity of glucose minimum intensity (GMI) to training adaptations. Despite a great use of glucose minimum intensity (GMI) and LMI in athletes population (MacIntosh et al., 2002; Simoes et al., 2003; Simoes et al., 1999; Sotero et al., 2009), if this protocol is not sensitive to training, the utilization for athletes can be questioned.

Thus, the aims of the present study were to analyze the sensitivity of LMI and GMI to changes in aerobic fitness resulting from endurance training. Our hypothesis is that both GMI and LMI are sensitive to endurance training and do not differ between each other.

### Methods

#### Participants

A group of eight trained cyclists took part in the study. All the athletes had at least two years of systematic training and participated in state and national competition. The mean  $\pm$ SD of age, body mass, height,  $VO_{2max}$  and training volume of the subjects were respectively  $21.4 \pm 1.9$  years,

$67.6 \pm 7.5$  Kg,  $1.72 \pm 0.10$  m,  $60.5 \pm 4.2$  ml·Kg<sup>-1</sup>·min<sup>-1</sup> and  $312.6 \pm 12.3$  km·week<sup>-1</sup>. The University's Institutional Review Board approved all procedures for human subjects (Human Research Ethics Committee). Athletes were briefed to the risks of the research prior to being given the opportunity to provide written informed consent.

### Experimental procedures

To evaluate the effect of training on LMI and GMI, the subjects completed two sets of tests, after the off-season period (March) and after 12 weeks of training (June):

- 1) A maximal incremental test in order to evaluate the maximal oxygen uptake (VO<sub>2</sub>max).
- 2) Test for assessment of LMI and GMI intensity.

The tests were performed at the same period of day ( $\pm 1$  h) to minimize diurnal variability (Dotan et al., 2011). All athletes performed the tests on a cycloergometer on different days and at least 24 hours apart. The seat height was selected by each subject and was the same on all trials. The inclusion criteria were: athletes who trained more than two years, and no recent history of injury. None of the subjects were diabetics. Before each test the athletes were instructed to avoid: any changes in nutritional habits, heavy training and caffeine ingestion during the 24-hour period preceding the test. Before the test the subjects consumed water ad libitum.

### Maximal oxygen consumption

Before the test, each athlete adjusted saddle height and the handlebars to his own cycling posture. The test was performed on a BIOTEC 2000 (CEFISE®) cycloergometric, and consisted of a 3-min constant workload warm-up at 50 W, after which power was increased by 25 W each minute until exhaustion. VO<sub>2</sub>max was determined as the highest value achieved over a 20-s period. VO<sub>2</sub> plateau, despite a power output increase, respiratory exchange ratio (RER) > 1.10 was used as criteria for VO<sub>2</sub>max achievement (Pardono et al., 2008; Seiler, 2010).

Respiratory parameters were continuously measured during the test from expired air using Vista CPX system (Vacumed®, 1996). Before each test the analyzers were calibrated. Oxygen uptake, carbon dioxide production and ventilation were measured every 30 seconds through Oxygen Analyzer OM-11, Carbon Dioxide Analyser LB-2 and Flow Transducer K – 520, respectively. Data was immediately processed by Vista CPX software. Heart rate was continuously monitored during the tests using a Polar f5 heart rate monitor (Polar Electro®, Finland)

### Glucose and lactate minimum intensities

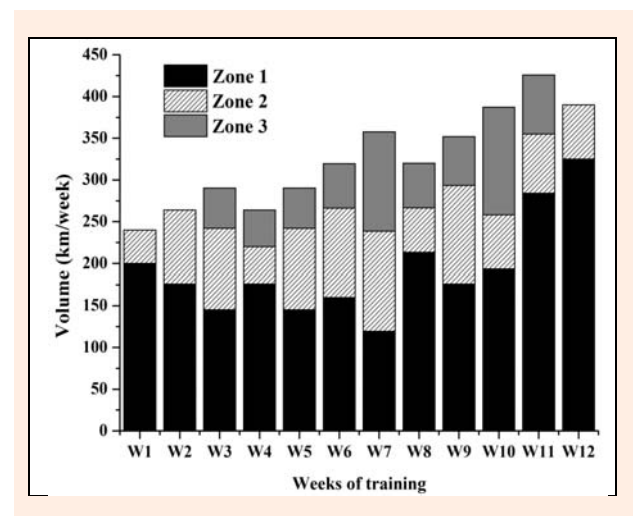
The GMI and LMI started with an all-out 30 seconds Wingate test (Bar-Or, 1987), with a 0.075 Kp/Kg body mass of loading for increase blood lactate. After eight minutes rest, each athlete started cycling at 125 W, with 25 W increases each three minutes, until voluntary exhaustion. In the end of each stage, blood was collected to measure BGI and BLA concentration. The pedaling cadence (RPM) was the preferred of the participant however; during incremental phase, they were oriented at maintain the cadence. The BGI and BLA curve showed a U-shaped feature and the GMI

and LMI was identified with a second order polynomial function, and the derivation of the equation (Simoes et al., 2010).

Blood samples were simultaneously taken from the athletes' earlobes and antecubital veins, seven minutes following the sprint and at each stage during the incremental test. Respiratory parameters were continuously measured during the incremental test. GMI and LMI were accessed both in watts and as percentage of VO<sub>2</sub>max. Heart rate was monitored during the tests using a Polar f5 heart rate monitor (Polar Electro®, Finland).

### Training program

Each intensity was divided according to heart rate (Seiler, 2010). The 12 weeks of training were prescribed in three different domains: intensity below 90% of HR relative to LMI (zone 1), intensity between 90% and 100% of HR relative to LMI (zone 2), and intensity above 100% of HR relative to LMI (zone 3). The "no-linear" or "undulating" periodization method was used in the period of the experiment (i.e., baseline period), this method consist in a progressive increase of volume with drastic variation of intensity during daily program (Issurin, 2010); the method the training was separated in 12 weeks with 240 km initial volume. The volume was increased every three week in  $\approx 10\%$ , except in the week four, eight and twelve, where the volume was the same of the second, sixth and tenth week, respectively (Figure 1).



**Figure 1.** Training intensity distribution for each twelve week of training. The bars indicate the volume swam in each intensity's zones. The filled bars indicate the volume at low intensity (zone 1); the dashed bars indicate the volume at medium intensity (zone 2); the filled gray bars indicate the volume at high intensity (zone 3).

Prescribe training intensity for professional road cyclists are difficult due to terrain modification, wind, and temperature. For this reason, the intensity was prescribed according to LMI heart rate. A Polar f5 heart rate monitor (Polar Electro®, Finland) controlled the HR. According to the target zone, when necessary increase of intensity of training, the cyclists was oriented to increase power, speed or maintain a sufficient slope to ensure the zone. Therefore, from the total distance covered at each training and the

response of heart rate, the total week volume in each zone was calculated.

The training distribution presents higher volume in the zone 1 (59.7%), 25% of training was at zone 2 and, 15.3% at zone 3. Almost all the weeks, the training week distribution was: Day 1 (zone 1), Day 2 (zone 2), Day 3 (zone 1), Day 4 (zone 3), Day 5 (zone 2), Day 6 (zone 1) and Day 7 (rest).

### Blood collection and laboratory analysis

For blood lactate determination, 25  $\mu$ l of blood sample was taken from the athletes' earlobe in calibrated capillaries and immediately placed and iced in Eppendorf tube containing 50  $\mu$ l 1% NaF solution for posterior electrochemical analysis (YLS 2700 STAT, Yellow Spring Co., USA). For blood glucose determination, 5 ml blood sample was taken from the athletes' antecubital vein continuously flushed with NaCl 0.85 % solution and immediately placed in polyethylene tubes containing 1% NaF, for posterior enzymatic analysis (oxidase method; COBAS Mira Plus; Roche Diagnostic System) (Iglay et al., 2007).

### Statistical analysis

The data are presented as mean and standard deviation (SD) of investigated variables. The Kolmogorov-Smirnov test was used to verify data normality. The comparison between pre and post training situations, the paired Student t test was used. The statistical significance was set at 5% and the analysis were performed using SPSS version 17.00 (SPSS Inc., Chicago, Illinois).

## Results

Post-training blood lactate level after maximal exercise ( $12.21 \pm 1.71$  mM) was significantly greater than in pre-training situation ( $11.71 \pm 1.60$  mM) ( $p < 0.05$ ). No significant difference was found in blood glucose level between

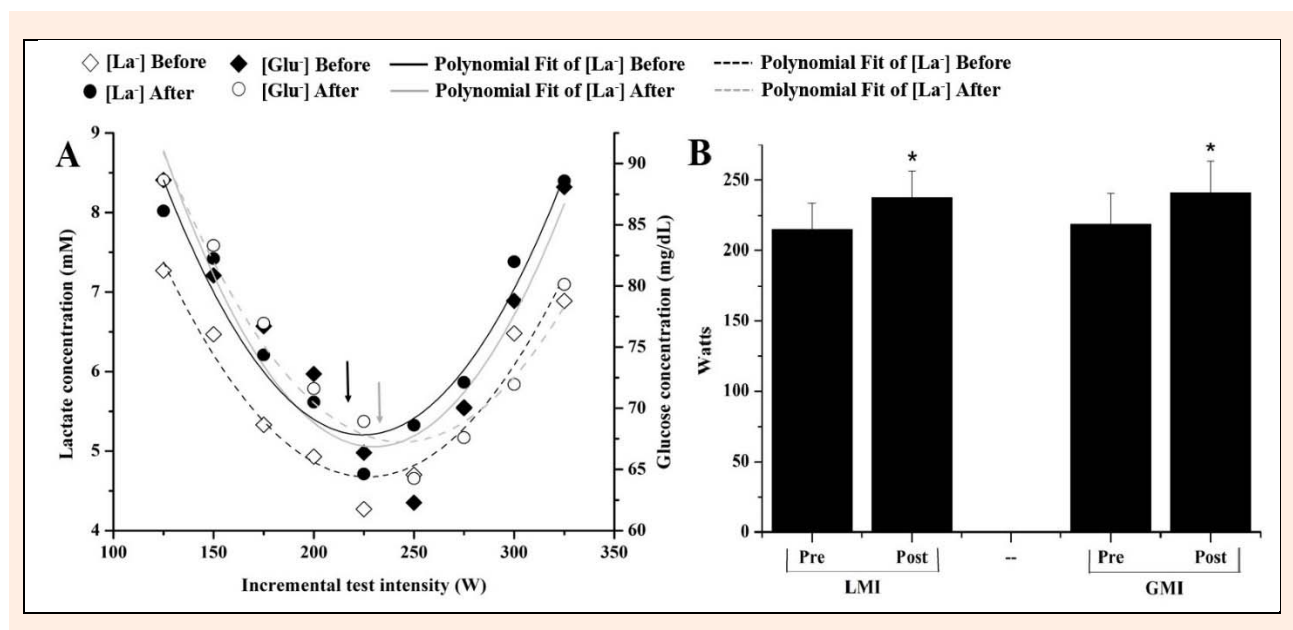
pre ( $90.37 \pm 2.99$  mg·dl<sup>-1</sup>) and post ( $91.50 \pm 3.02$  mg·dl<sup>-1</sup>) training, seven minutes after Wingate test.

No significant differences were observed between LMI and GMI (expressed both in Watts and %VO<sub>2</sub>max), neither between heart rates at these intensities both in pre and post training conditions (Table 1). VO<sub>2</sub>max, LMI and GMI increased significantly ( $p < 0.05$ ) after 12 weeks of endurance training ( $60.5 \pm 4.2$  vs.  $62.0 \pm 4.0$  ml·kg<sup>-1</sup>·min<sup>-1</sup>;  $215.0 \pm 18.6$  vs.  $237.5 \pm 18.8$  watts;  $218 \pm 22.1$  vs.  $240.6 \pm 22.9$  watts, respectively) (Table 1 and Figure 2). Additionally, the expected behavior of the LMI and GMI curves before and after 12 weeks of endurance training were present on Figure 2 as well.

**Table 1.** Mean ( $\pm$ SD) values for heart rate, lactate and glucose concentration associated to the LMI and GMI, and maximal oxygen uptake in pre and post training program.

|  | Variables                                 | Measures   | Mean ( $\pm$ SD) |
|--|---|------------|------------------|
| LMI                                      | %VO <sub>2</sub> max                      | Pre        | 71.5 (2.6)       |
|  |   | Post       | 72.6 (1.9) *     |
|  | HR (bpm)                                  | Pre        | 166 (1)          |
|  |   | Post       | 166 (1)          |
| BL <sub>MIN</sub> (mM)                   | Pre                                       | 4.2 (.5)   |                  |
|  | Post                                      | 4.3 (.5)   |                  |
| GMI                                      | %VO <sub>2</sub> max                      | Pre        | 71.8 (2.5)       |
|  |   | Post       | 73.0 (1.5) *     |
|  | HR (bpm)                                  | Pre        | 166 (1)          |
|  |   | Post       | 166 (1)          |
| BG <sub>MIN</sub> (mg·dl <sup>-1</sup> ) | Pre                                       | 64.6 (1.9) |                  |
|  | Post                                      | 64.2 (1.9) |                  |
| VO <sub>2</sub> max                      | (ml·kg <sup>-1</sup> ·min <sup>-1</sup> ) | Pre        | 60.5 (4.2)       |
|  |   | Post       | 62.0 (4.0) *     |

LMI = lactate minimum intensity; GMI = glucose minimum intensity; VO<sub>2</sub>max = maximal oxygen consumption; %VO<sub>2</sub>max = percentage of the intensity (LMI and GMI) corresponding to VO<sub>2</sub>max; HR = heart rate; BL<sub>MIN</sub> = lactate concentration corresponded to lactate minimum intensity; BG<sub>MIN</sub> = glucose concentration corresponded to glucose minimum intensity. \*significantly different from pre situation.



**Figure 2.** A) Expected behavior of the lactate and glucose minimum curves before and after a endurance training period and; B) Effect of training in GMI and LMI work load (W). \*significantly different from pre ( $p < 0.05$ ).

## Discussion

The aim of this study was analyze the effect of LMI and GMI after 12 weeks of training in cyclists. The main finding was that both LMI and GMI were sensitive to 12 weeks of training.

The lactate concentration after the Wingate test was significantly higher after training. Edge et al. (Edge et al., 2005) showed that 5-weeks high intensity training (120% - 140% of LTAN) improves repeated-sprint ability (5 X 6-s sprints, every 30 s) more than moderate intensity training (85% - 95% of LTAN). However, no difference was found between the groups of BLA after the repeated-sprint test. According to the authors, the higher ability on intermittent test after high intensity training is due to the capacity to maintain a high work output in the latter sprints, since no difference was found between groups in BLA.

Even with little time spent at intensities above LTAN, the aerobic training of the present study may have caused higher anaerobic energy supply during Wingate test. However, since this study did not present the baseline pre and post lactate level, this statement should be viewed with caution because higher baseline lactate level could determine the BLA- peak.

The LMT has been increasingly used in athletic evaluation, because it is an objective, rapid, and independent method of the muscle glycogen content for assess the LTAN during running. However, LMI seems to be affected by different factors, including initial speed (Carter et al., 1999b; Pardon et al., 2008) and stage length (Simoes et al., 2009) used during the graded phase of the test. Since the test is protocol dependent, its sensibility to endurance training was questioned (Carter et al., 1999a).

Johnson and Sharpe (Johnson and Sharpe, 2011) showed that one minute interval between LMI stages and initial intensity of 40% of maximal power output, decreased the power output of the test compared with a continuous test with initial intensity of 45% of maximal power output. To avoid this, our study used an initial power of 125 W with increment of 25 W that was a valid test to predict maximal lactate steady state (Johnson and Sharpe, 2011; Wahl et al., 2017).

Until now, Carter et al. (Carter et al., 1999a) were the unique study to test LMI sensitive to aerobic training. They questioned the sensitivity to endurance training adaptations. These authors showed LMI to be not changed after six weeks of aerobic training in 16 students, in spite of significant increases in  $\text{VO}_2\text{max}$ , running speeds at 3 mM blood lactate, lactate threshold and MLSS. Together, these results suggest that LMI depends on blood lactate kinetics during the test, which in turn is protocol dependent.

Our study is the first to find a significant increase in LMI after 12 weeks of training ( $215.0 \pm 18.6$ ,  $237.5 \pm 18.8$  watts;  $p < 0.05$ ), and it is in disagreement with Carter et al. (Carter et al., 1999a) which applied exactly the same exercise test protocols prior and after training and used active recovery after maximal effort. This resulted in lower blood lactate concentrations prior to the incremental phase of the test, in the post-training situation, due to (i) lower relative intensities of the supra-maximal bouts (from 120 to 111%  $\text{VO}_2\text{max}$ ) and (ii) higher lactate clearance during recovery

(Ribeiro et al., 2009). Considering the improvement of aerobic fitness with training, during the maximal exercise, the subjects presented less BLA- due to higher lactate clearance, oxidation and gluconeogenesis (Dotan et al., 2011). Maybe, during recovery the individuals could also present higher lactate clearance. Besides that, Ribeiro et al. (Ribeiro et al., 2009) showed that active recovery after maximal exercise, that induce hyperlactemia, underestimates the LMI.

The factors mentioned above would approach lactate concentration in the first series of incremental effort close to rest values, and become the U-shaped curve similar to single graded exercise test (Ribeiro et al., 2009), making it difficult to visualize the test sensibility to training. In concordance with these findings, Carter et al. (Carter et al., 1999a) pointed out that it is possible that LMI curve could have shifted to the right, if they had taken the increased fitness of the participants after the training program.

Since endurance training involves manipulation of intensity, duration, and frequency of training session over days (Seiler, 2010), comparison between this study and Carter et al. (Carter et al., 1999a) is difficult. Others methodological problems (i.e. training intensity and duration, sample characteristics) could also have minimized training adaptations, diminishing the sensibility. Together, these factors could partially explain the differences between ours and previous results (Carter et al., 1999a).

Once blood lactate testing is not always possible due to lack of equipment, great attention has been devoted to alternative methods for LTAN assessment (Dumke et al., 2006; Simoes et al., 1999; Van Schuylenbergh et al., 2004). The lowest serum glucose level was recently described as a good predictor of individual anaerobic threshold (IAT), LMI and MLSS for endurance runners (Simoes et al., 1999; Sotero et al., 2009).

Simões et al. (Simoes et al., 1999) found no significant differences between LMI, IAT speed and, GMI during track running tests. Recently, Sotero et al. (Sotero et al., 2009) also did not find differences between GMI and MLSS speed in physically active individuals (201.7  $\text{m}\cdot\text{min}^{-1}$  and 201.5  $\text{m}\cdot\text{min}^{-1}$ , respectively), and, concluded that GMI is a good predictor of the MLSS.

Recently Rocha et al. (Rocha et al., 2010) showed that individual glucose threshold changes in 12 weeks of military training, suggesting that this method could be useful for evaluating increases in aerobic fitness. According to Rocha et al. (Rocha et al., 2010), due to soldiers activities, they were not in adequate rest, although, this may not influenced the sensibility of the test. Since there, Rocha et al. (Rocha et al., 2010) found sensibility in individual glucose threshold, and this is the first study that showed sensibility in GMI, comparisons with other results are difficult due to lack of information.

From the observation of Simões et al. (Simoes et al., 1999), the hormonal modifications due to exercise may influence glucose availability, enabling the determination of GMI, and it seems that GMI is a good predictor for MLSS (Sotero et al., 2009). Maybe, a delay on hormonal release after training may contribute to alteration on GMI. Although its invasive nature, blood glucose testing presents



advantages over lactate related protocols, due to the lower cost and great number of existing analyzers. Consequently, it could be used by a large number of coaches, researchers and other professionals, both for research and training purposes.

The close occurrence of lactate and glucose minimum intensities during LMT may be explained by neural and hormonal alterations that take place in the transition from moderate to high intensity effort (Simoès et al., 1999). Increased sympathetic neural activity, as well as, the increased circulation of hormones is known for stimulating liver and muscle glycogenolysis (McGehee et al., 2005) that occur at supra LTAN intensities. This physiological event promotes lactate and glucose appearance to overcome their utilization rate by different tissues. However, more detailed studies are needed to investigate the physiological basis of glucose and lactate minimum existence during LMT.

The modification of LTAN and VO<sub>2</sub>max after aerobic training has already been demonstrated by others (Carter et al., 1999a; Edge et al., 2005; Enoksen et al., 2011; Rocha et al., 2010). Among the factors that might influence the aerobic adaptation are: enhance of monocarboxylate transporter proteins, increase of nicotinamide adenine dinucleotide reduced form shuttle enzymes levels, running economy and others (Enoksen et al., 2011; Ferrauti et al., 2010; Ziemann et al., 2011).

Although we have not examined the influence of training on more traditional lactate parameters, our results suggest that they could be successfully used to monitor training adaptations. However, more studies are needed to analyze the effects of training and protocol manipulation on both parameters. The LMT and GMT sensibility after off-season limits the study, since the LMI and GMI should be sensitive to training periods within the competitive season. Besides, the absence of baseline values of glucose and lactate prior and after the training and the reliability of these measures is a limitation of the present study.

## Conclusions

In conclusion, our results suggest that LMI and GMI are sensitive to changes in aerobic fitness after 12 weeks of training in elite cyclists after off-season. An important practical application of LMI parameters is the assessment of training derived adaptations. Furthermore, LMI and GMI can be used as important index to training prescription. Continuous training based on intensity below LMI and GMI (90 – 95%) with volume varying between 30 to 50 minutes, or interval training (3 – 4 bouts of 4 – 5 minutes with 4 – 10 minutes of recovery) based on intensity above LMI and GMI (10 to 20% above), is interesting for coaches. As the intensity during cycling training and exercise is not always available for athletes, the use of HR of LMI and GMI is an alternative method to training load control. Although both GMI and LMI are important index of aerobic fitness, they cannot be used to determine aerobic fitness. Other variables, such as, VO<sub>2</sub>max, running economy, and aerobic threshold, must also be analyzed. In addition, since the sensibility of LMI and GMI was verified

to prescribe training to athletes, studies should be addressed to other subjects, such as, recreational runners and cyclists.

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### Key points

- The lactate and glucose minimum intensities (GMI) can be used for monitoring training effects on cyclists
- Although both GMI and lactate minimum intensities are important index of aerobic fitness, they cannot be used to determine aerobic fitness.
- The polarized training was effective for improvements of maximal oxygen uptake on trained cyclists.

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