Anaerobic Capacity estimated in A Single Supramaximal Test in Cycling: Validity and Reliability Analysis

Willian Eiji Miyagi1,2, Rodrigo de Araujo Bonetti de Poli1,2, Marcelo Papoti3, Romulo Bertuzzi4 & Alessandro Moura Zagatto1,2

The aim was to verify the validity (i.e., study A) and reliability (i.e., study B) of the alternative maximal accumulated oxygen deficit determined using only supramaximal effort (MAODALT) to estimate anaerobic capacity (i.e., estimated by the gold standard maximal accumulated oxygen deficit method (MAOD)) during cycling. In study A, the effects of supramaximal intensities on MAODALT and the comparison with the MAOD were investigated in fourteen active subjects (26 ± 6 years). In study B, the test-retest reliability was investigated, where fourteen male amateur cyclists (29 ± 5 years) performed the MAODALT twice at 115% of the intensity associated to maximal oxygen uptake (iVO2max). MAODALT determined at 130 and 150% of iVO2max was lower than MAOD (p ≤ 0.048), but no differences between MAODALT determined at 100, 105, 110, 120 and 140% of iVO2max (3.58 ± 0.53L; 3.58 ± 0.59L; 3.53 ± 0.52L; 3.48 ± 0.72L; 3.52 ± 0.61L and 3.46 ± 0.69L, respectively) with MAOD (3.99 ± 0.64L). The MAODALT determined from the intensities between 110 and 120% of iVO2max presented the better agreement and concordance with MAOD. In the test-retest, the MAODALT was not different (p > 0.05), showed high reproducibility when expressed in absolute values (ICC = 0.96, p < 0.01), and a good level of agreement in the Bland-Altman plot analysis (mean differences ± CI95%: −0.16 ± 0.53L). Thus, the MAODALT seems to be valid and reliable to assess anaerobic capacity in cycling.

High-intensity efforts require a high demand of energy, mainly supplied by the non-oxidative metabolic processes. Thus, anaerobic capacity, which is regarded as the maximal amount of energy (i.e., ATP) that can be resynthesized through the phosphagen and glycolytic metabolic pathways, has been considered an important physiological performance determinant in these efforts. Anaerobic capacity evaluation is complex due to the lack of an existing method universally accepted as the gold standard; nevertheless the maximal accumulated oxygen deficit protocol (MAOD) has been considered the most accepted procedure to estimate this parameter. However, despite the scientific acceptance of MAOD to estimate anaerobic capacity, the practical application of this method is frequently infeasible due to the large expenditure of time required for its determination.

In 2010, Bertuzzi et al. proposed assessing the MAOD using only a supramaximal exercise test (MAODALT) in cycling at 110% of peak intensity measured in the graded exercise test (GXT). They assumed the MAODALT as the sum of the oxygen equivalents from the glycolytic (i.e., accumulated lactate during the effort) and phosphagen metabolisms (i.e., fast component of excess post-oxygen consumption - EPOCFAST). These authors reported that the MAODALT was not different from and significantly correlated with conventional MAOD (r = 0.78). In addition, besides the lower time required, the other advantage of MAODALT is the possibility of estimating singly the energy contribution engaged in non-mitochondrial pathways.

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1Laboratory of Physiology and Sport Performance (LAFIDE), São Paulo State University (Unesp), School of Sciences, Department of Physical Education, Bauru – SP, 17033-360, Brazil. 2Post-Graduate Program in Movement Sciences, São Paulo State University (Unesp), Institute of Biosciences, Rio Claro – SP, Brazil. 3School of Physical Education and Sport of Ribeirão Preto (EFEEERP), University of São Paulo (USP), 14049907 – Ribeirão Preto, SP, Brazil. 4Endurance Sports Research Group (GEDAE), School of Physical Education and Sport, University of São Paulo (USP), 05508030, São Paulo - SP, Brazil. Correspondence and requests for materials should be addressed to A.M.Z. (email: azagatto@yahoo.com.br)
In order to consolidate the MAODALT as an anaerobic capacity predictor, Zagatto et al. investigated its validity and reliability in running. The main arguments given for these authors to investigate the issues were the possible alterations in accumulated blood lactate concentrations ([La–]) and EPOC, due to different time-to-exhaustion at supramaximal intensities (MAODALT estimated at 100, 105, 110, 115, 120, 130, 140, and 150% of intensity) associated with the maximal oxygen uptake ([VO2max]) and to compare the MAODALT protocol with the MAOD determined by a gold standard method. The authors found that the MAODALT is a valid protocol to estimate anaerobic capacity and it is not altered by the supramaximal effort intensity. Furthermore, it was found that the ideal intensity to determine MAODALT was the velocity of 115% of VO2max (time-to-exhaustion 156.8 ± 7.6 s), which presented a high level of agreement and concordance with the conventional method (r = 0.73) as well as being reliable (i.e., test and retest intraclass correlation of 0.87).

On the other hand, there is a lack of studies investigating these issues with respect to MAODALT in cycling, since Bertuzzi et al. only investigated 110% in cycling while Zagatto et al. looked at a range of supramaximal efforts but during treadmill running. This is an intriguing issue because different physiological responses can be observed in cycling compared to running and MAOD is affected by the exercise mode. It is important to note that one of the main arguments that reinforce the validity of MAOD as a method for assessing anaerobic capacity is that the values remain constant when determined in supramaximal efforts lasting for 2 minutes. In situations of short time-to-exhaustion, MAOD values can be underestimated due to not reaching maximal capacity and an ideal time of between 2–3 minutes has been suggested to exhaust all capacity. However, it has been shown that all-out efforts lasting ~60 s can be used to determine MAOD, stressing the importance of investigating the supramaximal effort duration to estimate anaerobic capacity. Although Zagatto et al. have already investigated these issues concerning the MAODALT in running, it is necessary to know the possible effects in cycling as this ergometer is widely used in anaerobic evaluation. In addition, the importance of anaerobic capacity values expressed relative to active muscle mass has been documented, however for MAODALT this has not been reported.

Therefore, the main purpose of the present study was to verify the validity and reliability of the MAODALT in cycling. For this purpose, the following were investigated: 1) the effects of supramaximal intensities on MAODALT and the comparison with conventional MAOD; 2) the test-retest reliability. In accordance with the running results it was hypothesized that the MAODALT would not present differences from MAOD, would be independent of the supramaximal effort, and would be reproducible.

### Results

All subjects achieved at least two criteria to confirm maximal oxygen uptake (VO2max) determination (Table 1).

| Study | VO2max (mL·kg−1·min−1) | RER | HRmax (bpm) | RPE | [La–]peak (mmol·L−1) | Total time (min) |
|-------|------------------------|-----|-------------|-----|----------------------|-----------------|
| A     | 43.4 ± 4.9             | 1.20 ± 0.06 | 183 ± 6 | 18 ± 1 | 11.1 ± 1.4 | 12.2 ± 2.5 |
| (n = 14) | (40.5 to 46.2) | (1.17 to 1.23) | (180 to 187) | (17 to 19) | (10.3 to 11.9) | (10.8 to 13.6) |
| B     | 45.5 ± 7.4             | 1.21 ± 0.05 | 188 ± 9 | 18 ± 2 | 10.0 ± 2.1 | 12.8 ± 3.6 |
| (n = 11) | (40.5 to 50.5) | (1.17 to 1.25) | (181 to 194) | (17 to 20) | (8.5 to 11.5) | (10.4 to 15.3) |

Table 1. Physiological responses at exhaustion in the graded exercise test. Values presented as mean ± SD (CI95%). VO2max = maximal oxygen uptake. RER = respiratory exchange ratio. HRmax = maximal heart rate. RPE = rate of perceived exertion. [La–]peak = peak lactate.

The iVO2max was 280.1 ± 40.5 W (CI95% = 252.9 to 307.3 W). Table 4 shows the MAODALT values determined in the test and retest. No significant differences were found in MAODALT expressed in absolute or relative values (p > 0.05). In addition, significant correlations and a good level of agreement were found,
Table 2. Intensity, time to exhaustion, oxygen equivalent of the glycolytic metabolism (EiVO2max), resting lactate ([La−]rest), peak lactate ([La−]peak), difference between [La−]rest and [La−]peak (Δ[La−]), oxygen equivalent of the phosphagen metabolism (EPCr), amplitude (αi), and time constant (τi) of the bi-exponential adjustment obtained in the supramaximal efforts at 100, 105, 110, 120, 130, 140, and 150% of the intensity associated with maximal oxygen uptake (MAODALT100, MAODALT105, MAODALT110, MAODALT115, MAODALT120, MAODALT130, MAODALT140, and MAODALT150).

| Intensity (W) | 100% iVO2max | 105% iVO2max | 110% iVO2max | 115% iVO2max | 120% iVO2max | 130% iVO2max | 140% iVO2max | 150% iVO2max |
|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 225.6 ± 34.1 | 237.1 ± 35.8 | 248.5 ± 37.4 | 259.6 ± 39.3 | 270.8 ± 40.9 | 293.6 ± 44.2 | 315.9 ± 47.7 | 338.8 ± 51.0 | 620.62 |
| 105%         | 236.9 ± 27.0 | 252.7 ± 29.4 | 268.1 ± 31.2 | 284.8 ± 34.3 | 309.3 ± 36.8 | 338.8 ± 51.0 | 620.62 |
| 110%         | 226.9 ± 27.0 | 242.4 ± 29.7 | 258.1 ± 31.2 | 284.8 ± 34.3 | 309.3 ± 36.8 | 338.8 ± 51.0 | 620.62 |
| 115%         | 216.0 ± 27.0 | 232.7 ± 29.7 | 248.1 ± 31.2 | 274.8 ± 34.3 | 300.1 ± 36.8 | 328.8 ± 51.0 | 620.62 |
| 120%         | 206.0 ± 27.0 | 222.4 ± 29.7 | 238.4 ± 31.2 | 264.7 ± 34.3 | 290.2 ± 36.8 | 320.8 ± 51.0 | 620.62 |
| 130%         | 196.0 ± 27.0 | 212.4 ± 29.7 | 228.4 ± 31.2 | 254.7 ± 34.3 | 280.2 ± 36.8 | 310.8 ± 51.0 | 620.62 |
| 140%         | 186.0 ± 27.0 | 202.4 ± 29.7 | 218.4 ± 31.2 | 244.7 ± 34.3 | 264.7 ± 36.8 | 290.8 ± 51.0 | 620.62 |
| 150%         | 176.0 ± 27.0 | 192.4 ± 29.7 | 208.4 ± 31.2 | 234.7 ± 34.3 | 244.7 ± 36.8 | 270.8 ± 51.0 | 620.62 |

**Discussion**

The main finding of the study was that MAODALT was in general similar and statistically and significantly correlated with the MAOD. Despite the exercise at 130 and 150% of iVO2max leading to lower MAODALT values, its determination was not altered for 100, 105, 110, 120, 130, 140, and 150% of the intensity associated with maximal oxygen uptake (MAODALT100, MAODALT105, MAODALT110, MAODALT115, MAODALT120, MAODALT130, MAODALT140, and MAODALT150). Values presented as mean ± SD (IC95%), *p < 0.05 to 100% iVO2max, *p < 0.05 to 105% iVO2max, *p < 0.05 to 110% iVO2max, *p < 0.05 to 115% iVO2max, *p < 0.05 to 120% iVO2max, *p < 0.05 to 130% iVO2max, *p < 0.05 to 140% iVO2max.

Table 2 evidences the bias between the MAOD and MAODALT in nine healthy male subjects, reporting the validity of MAODALT to assess anaerobic capacity in cycling. These authors used six submaximal efforts to determine the MAOD, whilst using at least ten submaximal efforts has been suggested. In the present study, the MAOD was determined using ten submaximal efforts to construct the linear regression from the VO2 -intensity relationship, which reduces the random errors and increases the confidence in the determination of MAOD. Thus, the results showed that the MAODALT remains appropriate to estimate the MAOD, which is considered the most accepted technique to estimate the anaerobic capacity. Despite the relevant findings presented by Bertuzzi et al., these authors determined the MAODALT only from the intensity of 110% of peak intensity measured in GXT. Therefore, the use of different exercise intensities and the reliability in the test-retest analysis performed in the current study were used to solve some of these matters relevant to the practical use of the procedure, but under-investigated until the present date in cycling.

The lower values of MAODALT estimated at 130 and 150% of iVO2max are similar to those reported by Medbo et al., who verified that when the supramaximal effort is at least two minutes long the MAOD values remain constant, emphasizing the anaerobic capacity concept. Thus, similar differences observed at intensities above 120% of iVO2max (i.e., MAODALT120 and MAODALT130) in the present study could be related to these processes involved in the fatigue at intensities performed until exhaustion with short duration (i.e., less than two minutes). Possibly, this did not allow the maximal depletion of energetic substrate stores engaged in the glycolytic and phosphagen metabolic pathways. In addition, the power-duration relationship might be affected by the population. It is relevant to consider that as the MAODALT between 100 and 120% of iVO2max did not differ and were moderate and significantly correlated with MAOD, the time-to-exhaustion range varied around 164s (i.e., 2.74 ± 0.56 min for 120% of iVO2max) to 313s (i.e., 5.22 ± 0.99 min for 100% of iVO2max) could be used to determine the MAODALT in cycling. Despite the fact that MAODALT estimated at 140% of
iVO₂max did not differ from MAOD, this exercise intensity must be avoided, as the time-to-exhaustion varies around 110s (i.e., 1.83 ± 0.26 min) and impairs anaerobic capacity estimation due to acidosis-induced fatigue.

The issue of the normalization of anaerobic capacity values for muscle mass involved in the exercise appears to be an important factor to be considered. For example, Hill and Vilgren\(^1^6\) showed that the MAOD is sensitive to muscle mass involved in exercise, when comparing the values obtained in running and cycling. In this sense, some studies have investigated the use of MAOD values expressed relative to active muscle mass involved in cycling.\(^1^7,1^8\) In the present study, moderate (i.e., coefficient of correlations between 0.54 and 0.68) and significant correlations were observed in MAOD\(_{ALT}\) values expressed in absolute units and relative to lean mass of the lower limbs (LM-LL) (Table 3), but not when normalized by the total body mass or lean mass (LM). A possible explanation could be related to the differences between the two methods of determination of the anaerobic metabolism. The MAOD\(_{ALT}\) is based on the sum of the oxygen equivalents corresponding to glycolytic and phosphagen pathways estimated during the supramaximal effort.\(^5,8,1^9,2^0\) The latter takes into account an energetic equivalent expressed relative to the individual's body mass (i.e. mL·kg\(^{-1}\) of body mass). Therefore, the body mass of the individual will only be considered in the calculation when these values of oxygen equivalents are expressed in absolute units. On the other hand, values related to body mass could also influence the results because they include values of fat and bone mass, decreasing MAOD\(_{ALT}\) values of individuals with higher fat percentage. Likewise, results may be influenced when MAOD\(_{ALT}\) values are expressed relative to total lean mass because they include both active and inactive muscles. We observed that levels of association increased when MAOD values were expressed relative to LM and LM-LL, and statistical significance was only observed for the latter (Table 3). Even with moderate correlations observed, it is possible to suggest that this result perhaps provides more evidence indicating the validity of the protocol because it takes into account active muscle mass during exercise. Although moderately active individuals participated in the present study, another explanation for the results found in relation to normalization of MAOD and MAOD\(_{ALT}\) values could be related to possible differences in metabolic adaptations resulting from training. Pizza \textit{et al.}\(^1^7\) found that subjects undergoing resistance training presented higher MAOD values relative to lean mass of lower limbs than those trained in endurance and untrained individuals. These authors suggested that metabolic adaptations such as increased amounts of glycolytic and phosphagen enzymes may contribute to higher MAOD values, in addition to active muscle mass.

Figure 1. Comparison of the maximal accumulated oxygen deficit determined by the conventional method (MAOD) and alternative method (MAOD\(_{ALT}\)). The MAOD\(_{ALT}\) was determined at intensities of 100, 105, 110, 115, 120, 130, 140, and 150% of the intensity associated with maximal oxygen uptake (MAOD\(_{ALT100}\), MAOD\(_{ALT105}\), MAOD\(_{ALT110}\), MAOD\(_{ALT115}\), MAOD\(_{ALT120}\), MAOD\(_{ALT130}\), MAOD\(_{ALT140}\), and MAOD\(_{ALT150}\)). The MAOD and MAOD\(_{ALT}\) values are presented in absolute (A), relative to body mass (B), lean mass (LM) (C), and lean mass of lower limb values (LM-LL) (D). *p < 0.05 to MAOD.
### MAOD (L)

| Coefficient of correlation | MAOD<sub>ALT100</sub> | MAOD<sub>ALT105</sub> | MAOD<sub>ALT110</sub> | MAOD<sub>ALT115</sub> | MAOD<sub>ALT120</sub> | MAOD<sub>ALT130</sub> | MAOD<sub>ALT140</sub> | MAOD<sub>ALT150</sub> |
|----------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Mean of differences (CI95%) | (0.01 to 0.83)        | (0.06 to 0.85)        | (0.20 to 0.88)        | (0.24 to 0.89)        | (0.14 to 0.87)        | (0.03 to 0.84)        | (0.18 to 0.88)        | (0.12 to 0.86)        |
| Effect size                | 0.71                  | 0.67                  | 0.80                  | 0.75                  | 0.75                  | 1.03                  | 0.80                  | 0.96                  |
| Typical error (L)          | 0.41                  | 0.40                  | 0.35                  | 0.39                  | 0.39                  | 0.44                  | 0.40                  | 0.39                  |

### MAOD (mL·kg<sup>−1</sup>)

| Coefficient of correlation | MAOD<sub>ALT100</sub> | MAOD<sub>ALT105</sub> | MAOD<sub>ALT110</sub> | MAOD<sub>ALT115</sub> | MAOD<sub>ALT120</sub> | MAOD<sub>ALT130</sub> | MAOD<sub>ALT140</sub> | MAOD<sub>ALT150</sub> |
|----------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Mean of differences (CI95%) | (−0.12 to 0.79)       | (−0.34 to 0.68)       | (−0.41 to 0.63)       | (−0.30 to 0.70)       | (−0.31 to 0.70)       | (−0.66 to 0.37)       | (−0.32 to 0.69)       | (−0.58 to 0.47)       |
| Effect size                | 0.85                  | 0.97                  | 1.18                  | 1.13                  | 1.03                  | 1.78                  | 1.14                  | 1.78                  |
| Typical error (mL·kg<sup>−1</sup>) | 4.89                  | 5.27                  | 5.17                  | 5.46                  | 5.44                  | 6.05                  | 5.67                  | 5.30                  |

### MAOD (mL·kg<sup>−1</sup> LM)

| Coefficient of correlation | MAOD<sub>ALT100</sub> | MAOD<sub>ALT105</sub> | MAOD<sub>ALT110</sub> | MAOD<sub>ALT115</sub> | MAOD<sub>ALT120</sub> | MAOD<sub>ALT130</sub> | MAOD<sub>ALT140</sub> | MAOD<sub>ALT150</sub> |
|----------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Mean of differences (CI95%) | (−0.02 to 0.82)       | (−0.26 to 0.72)       | (−0.28 to 0.72)       | (−0.10 to 0.79)       | (−0.13 to 0.78)       | (−0.48 to 0.58)       | (−0.07 to 0.80)       | (−0.25 to 0.73)       |
| Effect size                | 0.78                  | 0.93                  | 1.17                  | 0.98                  | 0.92                  | 1.53                  | 0.96                  | 1.37                  |
| Typical error (mL·kg<sup>−1</sup> LM) | 6.63                  | 7.08                  | 6.75                  | 7.19                  | 7.16                  | 8.18                  | 7.42                  | 7.00                  |

### MAOD (mL·kg<sup>−1</sup> LM-LL)

| Coefficient of correlation | MAOD<sub>ALT100</sub> | MAOD<sub>ALT105</sub> | MAOD<sub>ALT110</sub> | MAOD<sub>ALT115</sub> | MAOD<sub>ALT120</sub> | MAOD<sub>ALT130</sub> | MAOD<sub>ALT140</sub> | MAOD<sub>ALT150</sub> |
|----------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Mean of differences (CI95%) | (0.13 to 0.87)        | (−0.10 to 0.80)       | (−0.14 to 0.78)       | (0.04 to 0.84)        | (−0.03 to 0.82)       | (−0.39 to 0.65)       | (0.08 to 0.85)        | (−0.23 to 0.74)       |
| Effect size                | 0.71                  | 0.81                  | 1.03                  | 0.90                  | 0.85                  | 1.45                  | 0.89                  | 1.36                  |
| Typical error (mL·kg<sup>−1</sup> LM-LL) | 18.10                 | 19.97                 | 19.03                 | 20.23                 | 20.28                 | 23.33                 | 20.63                 | 20.27                 |

### Table 3. Relationship and level of agreement between MAOD and MAOD<sub>ALT</sub> determined at different supramaximal efforts. *p < 0.05. **p < 0.01. LM-LL = lean mass of lower limbs.

The main findings of study B were that MAOD<sub>ALT</sub> determined from 115% of VO<sub>2</sub> max showed no significant differences in the test and retest (effect size between −0.06 and 0.24; coefficient of variation between 4.1 and 4.5%), high reproducibility (i.e., intraclass correlation coefficient between 0.81 and 0.96), and a good level of agreement (mean differences ± CI95%: −0.16 ± 0.53 L). The MAOD is an evaluation protocol used to estimate the anaerobic capacity that demonstrates reproducibility.<sup>4</sup> Weber and Schneider<sup>4</sup> investigated the reproducibility of the MAOD determined from the intensities of 110 and 120% of VO<sub>2</sub> peak and found high reproducibility (i.e., intraclass correlation coefficient of 0.95 and 0.97, respectively). In the same way, our findings for MAOD<sub>ALT</sub> are similar to those for running<sup>5</sup>, which showed that the MAOD<sub>ALT</sub> presented a mean difference very close to zero in the Bland-Altman analysis (Fig. 2), a small effect size (<0.2)<sup>21</sup>, and high intraclass correlation coefficient<sup>22</sup>, attesting that the MAOD<sub>ALT</sub> seems to be reliable and reproducible (Table 4). However, to our knowledge, this is the first study to investigate and demonstrate the reproducibility of MAOD<sub>ALT</sub> and the respective contributions of the glycolytic and phosphagen metabolisms in cycling.

Quantification of anaerobic capacity through the MAOD method should be reliable enough to allow evaluation and monitoring of possible changes related to training<sup>5</sup>. The MAOD<sub>ALT</sub> seems to be an advantageous tool for use during routine training because it allows the estimation of anaerobic capacity in only one supramaximal exercise session. Furthermore, the MAOD<sub>ALT</sub> allows discrimination of the glycolytic and phosphagen metabolisms, enabling analysis of the specific responses in each metabolism<sup>23</sup> and it can be considered a sensitive enough procedure to distinguish the "anaerobic" capacity in individuals with different training levels<sup>16</sup>. Regarding the reliability, although the points obtained from the differences between MAOD<sub>ALT</sub> values are distributed around the systematic error, there was a limit of agreement in test-retest corresponding to ±0.54L (Fig. 2). These values corresponded to ~10% of the mean values obtained for the MAOD<sub>ALT</sub> in study B. Thus, one should use caution when the MAOD<sub>ALT</sub> value is used, for example, to verify possible responses to training, especially if the pre- and post-training differences are less than these values. However, it has been shown that anaerobic capacity can be improved by 16<sup>24</sup> and 28%<sup>25</sup> with six weeks appropriate training in moderately active men. Nevertheless, it is important to note that the limits of agreement from the absolute and relative MAOD<sub>ALT</sub> values in the present study were lower than observed for the conventional MAOD in the test and retest condition<sup>26</sup> and when the MAOD<sub>ALT</sub> is used to estimate the conventional MAOD<sup>5</sup>. Nevertheless, another measure of reliability was also favorable, showing a low coefficient of variation of ~4%, which is lower than the findings for the conventional MAOD<sup>26,27</sup>.

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A possible limitation of the study was that although the MAOD ALT values obtained at other supramaximal intensities presented significant associations with MAOD, the reliability was only tested at 115% of $i\text{O}_{2\text{max}}$. Furthermore, the glycolytic pathway is based on blood lactate concentrations, which do not represent the exact stoichiometry between lactate formation and ATP resynthesis. As an alternative method, intramuscular metabolites could be used to quantify the anaerobic contribution during the exercise. The muscle tissue used for these measurements is usually obtained from small muscle mass. Consequently, it would be necessary to determine the muscle mass that is involved in a specific exercise, which could result in an inaccurate representation of anaerobic metabolism activation. 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.

Based on the findings of the current study, it is possible to reinforce that the MAOD ALT is a valid procedure to estimate the MAOD (i.e., anaerobic capacity) in cycling using only one supramaximal effort. The exercise intensities from 110 to 120% of $i\text{O}_{2\text{max}}$ provide the better results of the estimated MAOD by the MAOD ALT, mainly when values are expressed in absolute values. Further studies are encouraged to confirm the reliability of MAOD ALT determination using other exercise intensities that are also associated with conventional MAOD.

### Table 4.

| Measure | Test | Retest | ES | CV | p-value | ICC (CI95%) |
|---------|------|--------|----|----|---------|-------------|
| Time to exhaustion (s) | 182.1 ± 16.2 (171.2 to 193.0) | 180.7 ± 25.2 (163.8 to 197.7) | 0.17 | 4.5% | 0.708 | 0.981 (0.43 to 0.94) |
| MAOD ALT (L) | 4.42 ± 0.92 (3.80 to 5.03) | 4.58 ± 0.96 (3.94 to 5.23) | 0.24 | 4.1% | 0.01 | 0.96 (0.85 to 0.99) |
| MAOD ALT (mL·kg$^{-1}$) | 52.2 ± 7.5 (47.1 to 57.2) | 54.1 ± 8.2 (48.5 to 59.6) | 4.5% | 0.082 | 0.72 (0.72 to 0.98) |
| MAOD ALT (mL·kg$^{-1}$ LM) | 70.0 ± 8.7 (64.1 to 75.8) | 72.5 ± 9.5 (66.1 to 78.8) | 4.1% | 0.086 | 0.89 (0.63 to 0.97) |

### Figure 2.

Bland-Altman plot analysis with MAOD ALT determined in test and retest. The MAOD ALT values expressed in absolute (A), relative to body mass (B), lean mass (C), and lean mass of lower limb values (D).

A possible limitation of the study was that although the MAOD ALT values obtained at other supramaximal intensities presented significant associations with MAOD, the reliability was only tested at 115% of $i\text{O}_{2\text{max}}$. Furthermore, the glycolytic pathway is based on blood lactate concentrations, which do not represent the exact stoichiometry between lactate formation and ATP resynthesis. As an alternative method, intramuscular metabolites could be used to quantify the anaerobic contribution during the exercise. The muscle tissue used for these measurements is usually obtained from small muscle mass. Consequently, it would be necessary to determine the muscle mass that is involved in a specific exercise, which could result in an inaccurate representation of anaerobic metabolism activation. 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.

Based on the findings of the current study, it is possible to reinforce that the MAOD ALT is a valid procedure to estimate the MAOD (i.e., anaerobic capacity) in cycling using only one supramaximal effort. The exercise intensities from 110 to 120% of $i\text{O}_{2\text{max}}$ provide the better results of the estimated MAOD by the MAOD ALT, mainly when values are expressed in absolute values. Further studies are encouraged to confirm the reliability of MAOD ALT determination using other exercise intensities that are also associated with conventional MAOD.
Table 5. Characteristics of the participants. Values presented as mean ± SD. BM = Body mass. LM = lean mass. LM-LL = lean mass of the lower limbs. VO₂max = maximal oxygen uptake. *Estimated by the dual-energy X-ray absorptiometry method.

| Study   | Age (years) | Height (cm) | Body Mass (kg) | Lean Mass (kg) | Fat (%) | LM-LL (kg) |
|---------|-------------|-------------|----------------|----------------|---------|------------|
| A (n = 14) | 26 ± 6      | 172.4 ± 5.4 | 73.8 ± 10.8    | 55.4 ± 6.4     | 19.4 ± 4.5 | 19.1 ± 2.4 |
| B (n = 11) | 28 ± 4      | 177.0 ± 5.8 | 85.2 ± 16.1    | 63.2 ± 11.0    | 20.5 ± 4.8 | 22.5 ± 4.4 |

Material and Methods

Subjects. In study A, eighteen moderately active men volunteered to participate. They reported practicing different physical activities such as resistance training, running, cycling, futsal, soccer, Brazilian jiu-jitsu, and swimming and they were not included in any systematic training. The subjects who presented repeated absences (3 subjects) or injuries (1 subject) were excluded. Thus, fourteen individuals completed study A. In study B, eleven mountain-bike cyclists, recreationally trained, volunteered to participate in the study. They reported at least six months experience with a training volume of 121.5 ± 48.5 km·week⁻¹ and training sessions three times per week. Table 5 presents the characteristics of the subjects of studies A and B. We used a sample of moderately active men in study A instead of trained individuals due to the difficulty in scheduling several visits of cyclists (i.e., around 10 visits in Study A) to the laboratory to perform the exercise bouts.

All subjects were instructed to avoid alcohol and caffeine ingestion and not to carry out exhaustive exercises for at least 24 h before each session.

We conducted the experiment according to the current International laws. The study was approved by the Ethics Committee of the Sao Paulo State University (Protocol 645.784/2014) and the study was conducted according to the Declaration of Helsinki. Informed consent was obtained from all participants.

Experimental design. All tests were performed on a cycle ergometer (Lode-Excalibur Sport, Lode BV, Groningen, The Netherlands) with free choice of cadence between 70–90 rpm, defined in a previous familiarization, as well as saddle settings, and crank distance from handlebars. Subjects were instructed to maintain the preferred cadence with a maximum variation of ±5 rpm in all evaluations. The procedures in each study were applied in an environment with controlled temperature and humidity (20 ± 1 °C and 61 ± 8%, respectively).

In both studies, the subjects were initially submitted to body composition evaluation and a graded exercise test (GXT) to determine the maximal oxygen uptake (VO₂max) and the associated intensity (iVO₂max). Then, in study A the individuals performed ten submaximal efforts (30–80% of iVO₂max) and eight supramaximal efforts (100–150% of iVO₂max). Each session was composed of one submaximal effort, which was used as a warm-up, and one supramaximal effort performed until exhaustion. Thus, eight MAODALT values were determined from the supramaximal efforts.

In study B, after the GXT, the individuals performed two supramaximal efforts at the intensity that presented the greatest level of agreement between MAOD and MAODALT determined in study A. The interval between each maximal test in both studies was 48 h.

Prior to the beginning of each exhaustive exercise session a POMS questionnaire was applied (i.e., Profile of Mood State)²⁵ to evaluate the mood state in order to ensure the best performance in efforts until exhaustion.

Physiological measurements. The oxygen uptake (VO₂) was measured breath-by-breath during all effort tests by a stationary gas analyzer, Quark PFT (COSMED, Rome, Italy). The gas analyzer was calibrated immediately before each test with known samples (5.0% CO₂ and 16.0% O₂, White Martins®, Osasco, Brazil) and room air, while the turbine was calibrated through a three-liter syringe (Hans-Rudolf, USA) following the manufacturer’s recommendations. The VO₂ values were smoothed each 5 points and interpolated each 1 second through OriginPro 9.0 software (OriginLab Corporation, Microcal, Massachusetts, USA).³⁵ The heart rate (HR) was measured by a transmitter belt coupled to the gas analyzer (Wireless HR 138 Monitor, COSMED, Roma, Italy) and the rate of perceived exertion (RPE) using the 6–20 Borg scale.³¹ Blood lactate ([La⁻]) was measured by blood samples (25 μL) collected from the ear lobe at rest and 3, 5, and 7 minutes after each maximal test to determine the peak lactate concentration ([La⁻]ₚ). The blood samples were collected and stored in Eppendorf tubes containing 50 μL of 1% sodium fluoride, stored in a freezer at −20 °C and then analyzed in an electrochemical lactometer YSI 2300 STAT (Yellow Spring Instruments, Ohio, USA). In addition, before each session, the subjects remained seated for ten minutes to determine the VO₂ and blood lactate at rest ([La⁻]₀). The resting VO₂ was considered the mean of the final two minutes.

Body composition analysis. Body composition was measured before all procedures by dual-energy X-ray absorptiometry (DXA) using the Discovery corporal scanner (Hologic, Sunnyvale, USA). The subjects were scanned and the analysis followed the manufacturer’s recommendations. The body segmentation analysis was carried out with the horizontal line positioned above the bowl slightly above iliac crest. The angular lines that define the pelvic triangle were sectioned at the femur, and the vertical line positioned between the legs dividing the two feet. The lean mass of the lower limbs (LM-LL) was considered the sum of the right and left legs, not considering the bone mass values.⁴⁴
Study A procedures

**Effect of supramaximal effort intensity on MAOD_{ALT}**. Graded exercise test (GXT). The GXT were carried out to reach exhaustion in ~8–12 minutes. The initial intensity was 75–100 W, with increments of 25 W each 2 minute stage until voluntary exhaustion or the inability to maintain the pre-set cadence. In each exercise stage, the VO_{2} measured during the final 30 s of the stage was averaged. Therefore, the highest average of VO_{2} obtained during the test was considered as VO_{2max}, following the criteria to confirm VO_{2max} determination.

The intensity associated with VO_{2max} (i\text{VO}_{2max}) was assumed as the lowest intensity where the VO_{2} was reached.

Submaximal and supramaximal efforts. The subjects performed ten submaximal efforts lasting ten minutes on different days (30, 35, 40, 45, 50, 55, 60, 65, 70 and 80% of i\text{VO}_{2max}) to construct the linear regression from the VO_{2}-intensity relationship (VO_{2}). For the higher exercise intensities (i.e., 70 and 80% of i\text{VO}_{2max}), only the effort where a VO_{2} steady state was observed was considered to avoid considering points of the VO_{2}-intensity relationship above the anaerobic threshold. The linear regression allowed the estimative of the supramaximal intensity demand and subsequently MAOD determination. The lowest submaximal effort was carried out together with the highest supramaximal effort. Consequently, the second lowest submaximal exercise was combined with the second highest intensity supramaximal exercise and so on. A 10 minutes rest interval was allowed between the submaximal and supramaximal efforts in each session. The supramaximal efforts at 70 and 80% of i\text{VO}_{2max} were performed separately so as not to directly affect the supramaximal efforts.

Each subject performed, in different sessions, eight supramaximal efforts until exhaustion at 100, 105, 110, 115, 120, 130, 140 and 150% of i\text{VO}_{2max}. The time to exhaustion was evaluated and the VO_{2} monitored at rest, during the test, and seven minutes after the test, for EPOCFAST analysis. Eight MAOD_{ALT} values were determined, corresponding to each supramaximal effort. The supramaximal intensity at 110% of i\text{VO}_{2max} was used to determine the MAOD.

**MAOD determination**. Initially, the linear regression was constructed from the relationship between the submaximal efforts and respective VO_{2}. The y-intercept was fixed with the mean of VO_{2} baseline values (rest) (Fig. 3A). The VO_{2} of each exercise intensity was assumed as the VO_{2} mean of the final two minutes of exercise. To estimate the supramaximal energetic demand, the linear regression was constructed to minimize possible effects related to the number of submaximal efforts, a fixed y-intercept and the non-linearity caused by the slow component of the VO_{2} when exercise intensities above the anaerobic threshold were included.

The supramaximal effort of 110% of i\text{VO}_{2max} was performed by the subjects and the MAOD corresponded to the difference between the area of the estimated VO_{2} supramaximal (product between the estimated demand and time to exhaustion) and accumulated VO_{2} during the test (Fig. 3B). The VO_{2} accumulated during the test was calculated using the trapezoidal method. The MAOD values were reduced by 10%, corresponding to the body oxygen stores for energy supply.

**Alternative MAOD (MAOD_{ALT})**. The MAOD_{ALT} corresponded to the sum of the oxygen equivalents from the phosphagen (E_{PCr}) and glycolytic (E_{[La]}) metabolisms, which were calculated in each session at 100 (MAOD_{ALT100}), 105 (MAOD_{ALT105}), 110 (MAOD_{ALT110}), 115 (MAOD_{ALT115}), 120 (MAOD_{ALT120}), 130 (MAOD_{ALT130}), 140 (MAOD_{ALT140}), and 150% (MAOD_{ALT150}) of i\text{VO}_{2max}. The MAOD_{ALT} values were presented in absolute values, relative to lean mass (LM), and lean mass of the lower limbs (LM-LL).

The E_{PCr} was assumed as the EPOCFAST area, calculated using a bi exponential adjustment (Equation 1), and corresponding to the product between amplitude 1 (A_{1}) and time constant1 (\tau_1) (Equation 2). The E_{[La]} was estimated by the difference between the peak and resting blood lactate concentrations, considering the oxygen equivalent of 3 mL·kg^{-1}·min^{-1} of accumulated lactate above resting level.

**Figure 3.** Supramaximal oxygen uptake (VO_{2}) estimated at 110% of the intensity associated with the maximal oxygen uptake (i\text{VO}_{2max}) (A) and the maximal accumulated oxygen deficit (MAOD) (B).
\[ \text{VO}_2(t) = \text{VO}_2(\text{Rest}) + A_1 e^{-\frac{t}{\tau_1}} + A_2 e^{-\frac{t}{\tau_2}} \]  

\[ E_{PCr} = A_1 \cdot \tau_1 \]  

where \( \text{VO}_2(t) \) is the oxygen consumption at time \( t \), \( \text{VO}_2(\text{Rest}) \) is oxygen consumption at rest, \( A \) is the amplitude, and \( \tau \) is the time constant. 1 and 2 mean the fast and slow components, respectively.

**Study B procedures.** MAOD\(_{ALT}\) Reliability. In Study B, the subjects performed the body composition evaluation, GXT, and two supramaximal efforts. The supramaximal efforts were carried out at the intensity that necessary. In addition, the comparison between MAOD and MAOD\(_{ALT}\) was performed with the effect size calculation, Greenhouse-Geisser Epsilon correction was used. The ANOVA was completed with the Sidak comparison test, if necessary. In addition, the comparison between MAOD and MAOD\(_{ALT}\) was performed with the effect size calculation (\( r^2 \)), Bland-Altman analysis\(^{40}\), Pearson's correlation test, and typical error. The coefficient of correlation was classified as very weak to negligible (0 to 0.2), weak (0.2 to 0.4), moderate (0.4 to 0.7), strong (0.7 to 0.9), and very strong (0.9 to 1.0)\(^{41}\). In study B, for comparison of the values obtained in the test and retest the test for repeated samples was used. The intraclass correlation coefficient (ICC (3.1); Two-way mixed, consistency, single measures) was used for analysis of reproducibility and Bland-Altman analysis to determine the level of agreement. In addition, the coefficient of variation was calculated by dividing the standard deviation and mean of each participant in the test-retest condition, and then calculating the mean for all subjects. IBM® SPSS 20 software (Chicago, IL, USA) was used for statistical analysis and in all cases a 5% level of significance was considered.

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Author Contributions

W.E.M. conducted the experiment, analyzed the results and wrote the manuscript; R.A.B.P. assisted in analysis and data collection; M.P. analyzed the results and wrote the manuscript; R.C.M.B. analyzed the results and wrote the manuscript; A.M.Z. conceived the experiment, analyzed the results and wrote the manuscript; all authors reviewed the manuscript.

Additional Information

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