

Maximal Oxygen Uptake, Muscular Oxidative Capacity, and Ventilatory Threshold in Amateur Triathletes: Eight-Month Training Follow-Up

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Purpose: Endurance sports performance is influenced by several factors, including maximal oxygen uptake ($\dot{V}O_{2\max}$), the percentage of $\dot{V}O_{2\max}$ that can be sustained in endurance events, running economy, and body composition. Traditionally, $\dot{V}O_{2\max}$ can be measured as an absolute value, adjusted for body mass, reflecting the athlete's central capacity (maximal cardiac output), or adjusted for lean mass (LM), reflecting the athlete's peripheral capacity (muscular oxidative capacity). The present study aims to evaluate absolute, total body mass, and lower limb LM-adjusted $\dot{V}O_{2\max}$, ventilatory thresholds (VT), respiratory compensation points (RCP), and body composition during two training periods separated by 8 months.

Patients and Methods: Thirteen competitive amateur triathletes [seven men (40.7±13.7 years old, 76.3±8.3kg, and 173.9±4.8cm) and six women (43.5±6.9 years old, 55.0±2.7kg, 164.9±5.2cm)] were evaluated for body composition with dual-energy X-ray absorptiometry and $\dot{V}O_{2\max}$, VT, RCP, and maximal aerobic speed (MAS) with a cardiorespiratory maximal treadmill test.

Results: The absolute $\dot{V}O_{2\max}$ ($p = 0.003$, $d = 1.05$), body mass-adjusted $\dot{V}O_{2\max}$ ($p < 0.001$, $d = 1.2859$), and MAS ($p = 0.047$, $d = 0.6139$) values differed significantly across evaluation periods. Lower limb LM-adjusted $\dot{V}O_{2\max}$ ($p = 0.083$, $d = -0.0418$), % $\dot{V}O_{2\max}$ at VT ($p = 0.541$, $d = -0.1746$), speed at VT ($p = 0.337$, $d = -0.2774$), % $\dot{V}O_{2\max}$ at RCP ($p = 0.776$, $d = 0.0806$), and speed at RCP ($p = 0.436$, $d = 0.2234$) showed no difference.

Conclusion: The sensitivities of $\dot{V}O_{2\max}$ adjusted for body mass and $\dot{V}O_{2\max}$ adjusted for LM to detect changes in physical training state differ. Furthermore, decreases in physical fitness level, as evaluated by $\dot{V}O_{2\max}$ values, are not accompanied by changes in VT.

Keywords: amateur triathletes, skeletal muscle oxidative capacity, triathlon, performance

Introduction

Endurance sports performance is determined by maximal oxygen uptake ($\dot{V}O_{2\max}$), the percentage of $\dot{V}O_{2\max}$ that can be sustained, and running economy.¹ Given the importance of $\dot{V}O_{2\max}$ and its association with endurance performance,² it has become one of the most investigated variables in exercise physiology. The absolute oxygen uptake rate per unit of time ($L \cdot \min^{-1}$) is measured as $\dot{V}O_{2\max}$. However, adjusting for total body mass is recommended when demonstrating cardiorespiratory fitness and comparing individuals.³ Elite endurance athletes may present more than $80 \text{ mL} \cdot \min^{-1} \cdot \text{kg}^{-1}$ and patients with cardiovascular disease less than $20 \text{ mL} \cdot \min^{-1} \cdot \text{kg}^{-1}$ values for $\dot{V}O_{2\max}$.³ In this context, the effects of training have been commonly assessed by $\dot{V}O_{2\max}$ adjusted to total body mass.⁴ Although $\dot{V}O_{2\max}$ is dependent on the integrated functioning of the respiratory, cardiovascular, and skeletal muscle systems at maximal exercise intensity,⁵ there

is a consensus that the main limiting factor for $\dot{V}O_{2\max}$ in healthy individuals is the maximum cardiac output, not the pulmonary or muscular skeletal systems.⁶

Maximal oxygen uptake can also be adjusted based on skeletal muscle mass, and researchers have also investigated this measurement in endurance-trained athletes once it reflects different physiological characteristics.³ Skeletal muscle mass adjusted $\dot{V}O_{2\max}$ represents the muscle's ability to consume oxygen, often known as "aerobic muscle quality".³ While absolute and body mass-adjusted $\dot{V}O_{2\max}$ are primarily limited by central conditions (maximum cardiac output), LM adjusted $\dot{V}O_{2\max}$ is limited mainly by peripheral conditions (skeletal muscle mass characteristics), and both variables can be used to demonstrate training or detraining effects. Moreover, the percentage of $\dot{V}O_{2\max}$ that can be sustained also has been considered an important determinant factor of endurance performance. As with LM adjusted $\dot{V}O_{2\max}$, the percentage of $\dot{V}O_{2\max}$ that may be sustained is primarily limited by adaptations in skeletal muscles caused by aerobic exercise training.⁷

Determining whether performance measures (such as absolute, adjusted $\dot{V}O_{2\max}$, and the percentage of $\dot{V}O_{2\max}$ that can be sustained) are similarly sensitive to identifying changes in training status might help coaches evaluate athletes and use these indices for exercise prescription. However, to the best of our knowledge, no previous studies have examined the changes in these variables between two different periods of training periodization over a long period. Therefore, the present study aims to evaluate absolute, total body mass and lower limb LM adjusted $\dot{V}O_{2\max}$, ventilatory thresholds (percentage of $\dot{V}O_{2\max}$ that can be sustained during endurance exercise), and body composition between two training periods separated by 8 months. We hypothesize that when different physiological factors influence each of these variables, the sensitivity in identifying changes in training status will not be equal for $\dot{V}O_{2\max}$ values adjusted for absolute body mass (BM) and lower limb LM (LLM).

Materials and Methods

Participants

Amateur triathletes of both sexes, between the ages of 26 and 60 participated in the study. They were recruited to participate in this study via social media (WhatsApp and Instagram). The inclusion criteria were age from 18 to 60 years old, at least 2 years of experience in the modality (triathlon), and no medical restrictions to maximal effort. Exclusion criteria included having to take a 30-day break from training, had underlying cardiovascular, respiratory, or muscular health conditions, suffered from acute musculoskeletal injuries, were pregnant, and failed to undergo laboratory testing for any reason. The initial evaluation tests were performed by 40 athletes in March 2022. The second evaluation occurs in November 2022, 1 month after the target race (at the end of September or beginning of October). Fourteen participants met the inclusion criteria and returned for retests. The most common reasons for follow-up losses were acute injury ($n = 4$), unavailability ($n = 2$), personal reasons ($n = 5$), no response after contact ($n = 13$), and a change in contact number ($n = 2$). After data collection, one participant was excluded from the study due to inconsistencies in cardiac monitoring, leaving 13 final participants from 26 to 60 years old, seven males (40.7 ± 13.7 years old, 76.3 ± 8.3 kg, 173.9 ± 4.8 cm) and six females (43.5 ± 6.9 years old, 55.0 ± 2.7 kg, 164.9 ± 5.2 cm) (Figure 1). There were no participants with less than 12 months of triathlon experience, 15.4% ($n = 2$) with 12 to less than 24 months, 23.1% ($n = 3$) with 24 to less than 36 months, and 61.5% ($n = 8$) with more than 36 months. All the participants were involved with resistance and triathlon training.

In terms of swimming, 53.8% ($n = 7$) trained 1 to <3 h/week, 46.1% ($n = 6$) 3 to <5 h/week, and nobody trained 5 to <7 h/week or more. In terms of cycling training, none trained 1 to <4 h/week, 46.1% ($n = 6$) 4 to <7 h/week, 53.8% ($n = 7$) 7 to <9 h/week, and nobody trained more than 9 h/week. According to Table 1, in terms of running, 61.5% ($n = 8$) trained 1 to <4 h/week, 38.4% ($n = 5$) 4 to <7 h/week, 53.8% ($n = 7$) 7 to <9 h/week, and nobody trained more than 9 h/week. All the participants were training for long-distance triathlon races during the period (Half or Full Ironman distance, that is 3800 swimming, 180km cycling, and 42 running).

Experimental Procedures

This was a longitudinal and observational study of amateur triathletes. The study adhered to the Declaration of Helsinki and the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals. The study was approved by the Human Ethics Committee of the University Federal of São Paulo-UNIFESP (approval number 5.059.538 on October 25, 2021). Participants were informed about the research objectives, proposed physiological laboratory tests, and

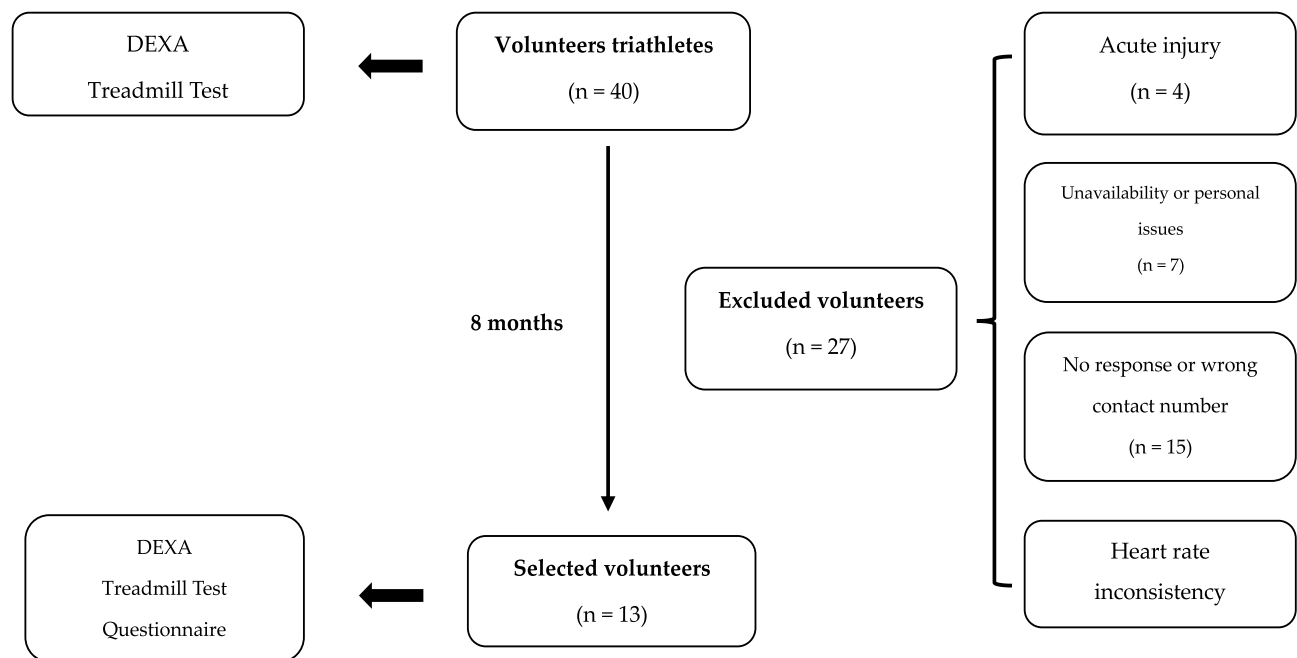


Figure 1 Study flowchart.

associated risks and benefits, with the researchers ensuring that principles of respect, privacy, confidentiality, and anonymity were preserved. Informed consent forms were obtained from all participants. Following that, the participants proceeded to the UNIFESP Exercise Physiology Laboratory twice to have their height, BM, and body composition measured. The participants were then subjected to a maximal treadmill test to determine their cardiorespiratory function. They were instructed not to engage in strenuous physical activity within 24 h of the test and not to consume stimulating foods, such as coffee, on the test day. Additionally, the researchers recommended that participants wear comfortable running shoes and lightweight clothing for the test.

The participants were evaluated twice at different times. The first evaluation occurred during the pre-competition period (March 2022), and the second occurred after the target race period (November 2022) for each participant, with an average interval of 8.7 ± 0.5 months. A retrospective questionnaire was included during the second evaluation to collect retrospective information on training, race participation, and health status between the two assessments.

Table 1 Characteristics of the Participants

Questions	Answer I	Answer I	Answer I	Answer I
Triathlon experience (months)	0 to < 12 0% (n = 0)	12 to < 24 15.4% (n = 2)	24 to < 36 23.1% (n = 3)	≥ 36 61.5% (n = 8)
Race distance played during 2022	Short or Sprint 0% (n = 0)	Olympic 76.9% (n = 10)	70.3 84.6% (n = 11)	Iron Man 46.1% (n = 6)
Target race of period	Short or Sprint 0% (n = 0)	Olympic 0% (n = 0)	70.3 53.8% (n = 7)	Iron Man 46.1% (n = 6)
Strength training (h/week)	1 to < 3 h 7.7% (n = 1)	3 to < 5 h 76.9% (n = 10)	5 to < 7 h 7.7% (n = 1)	≥ 7 h 7.7% (n = 1)
Swimming training (h/week)	1 to < 3 h 53.8% (n = 7)	3 to < 5 h 46.1% (n = 6)	5 to < 7 h 0% (n = 0)	≥ 7 h 0% (n = 0)
Cycling training (h/week)	1 to < 4 h 0% (n = 0)	4 to < 7 h 46.1% (n = 6)	7 to < 9 h 53.8% (n = 7)	≥ 9 h 0% (n = 0)
Running training (h/week)	1 to < 4 h 61.5% (n = 8)	4 to < 7 h 38.4% (n = 5)	7 to < 9 h 0% (n = 0)	≥ 9 h 0% (n = 0)

Questionnaire

During the second assessment, a retrospective questionnaire was administered, which included targeted questions about the duration of triathlon participation, the number and length of races completed, the primary/target race (and its distance) during the period, and the amount of time spent each week on swimming, cycling, and running exercises. Participants were also asked about any withdrawal from athletics, underlying health conditions, or medication use between evaluations ([Annex 1](#)). A response summary is presented in [Table 1](#).

Body Composition and Anthropometry

Body mass and height were measured with a precision of 0.1 kg and 0.1 cm, respectively, using a calibrated stadiometer Filizola® PL (Filizola, São Paulo, Brazil). Body composition (fat-free and fat mass) was evaluated using the dual-energy X-ray absorptiometry (DXA, software version 12.3, Lunar DPX, Wisconsin, USA). This method provides a rapid and non-invasive assessment of fat (FM) and fat-free mass with low radiation exposure and is a reference method in clinical research for determining body composition.^{8,9} Tests were performed with the participants in a supine position, wearing comfortable clothing, with no metal objects, centrally aligned with 10 cm between the feet and 5 cm between the hands and trunk, and performed after bladder emptying.¹⁰ The DXA was calibrated before each test according to the manufacturer's instructions.

Cardiorespiratory Maximal Treadmill Test

A cardiorespiratory maximal treadmill test (Inbrasport, ATL, Porto Alegre, Brazil) was performed by the participants. A computer-based system for analyzing breath-based gas exchange (Quark, Comed, Italy) was used to determine $\dot{V}O_2$ max, VT, RCP. This equipment presents a good accuracy.¹¹ The metabolic analyzer was calibrated before each test using the manufacturer's recommended procedures. On the day of the tests, participants were encouraged to avoid vigorous exercise, coffee, or stimulating foods. All the tests were performed during the morning period, by an experienced physician under the supervision of the principal investigator who has over 20 years of experience in cardiorespiratory maximal treadmill test, at room temperature of $20 \pm 2^\circ\text{C}$ and relative air humidity between 50% and 70%.¹²

The test began with a 3-min warm-up period at 8 km/h, followed by 1-min increments of 1 km/h until voluntary exhaustion was reached. The total test duration for all participants ranged from 8 to 12 min. To simulate the challenges of outdoor running, the treadmill was kept at 1% inclination throughout the test.¹³ Each stage ended with the Borg Rating of Perceived Exertion¹⁴ applied, and the heart rate (HR) was monitored using a HR monitor (Ambit 2S, Suunto, Finland).

The oxygen uptake ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), oxygen end-tidal pressure ($PETO_2$), carbon dioxide pressure ($PETCO_2$), and minute ventilation ($\dot{V}E$) were measured breath by breath. Data averages were calculated at 20-s intervals. The $\dot{V}O_2$ max was defined as the point at which oxygen consumption stabilized (or increased by less than $2.1 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$) even while the treadmill speed was increased.¹⁵ VT and RCP were identified using the O_2 and CO_2 ventilatory equivalents ($\dot{V}E/\dot{V}O_2$ and $\dot{V}E/\dot{V}CO_2$) and $PETO_2$ and $PETCO_2$ by two independent investigators.¹⁶ Speed and the $\dot{V}O_2$ max percentage were calculated at VT and RCP. The maximal aerobic speed (MAS) was measured and defined as the lowest exercise intensity resulting in $\dot{V}O_2$ max.

Statistical Analysis

The sample size was calculated using the G*Power version 3.1.9.7 (Franz, Universität Kiel, Germany). A total sample size of 15 individuals was determined for an effect size of 0.7, type I error of 5%, and type II error of 20%. Data were presented as the mean and standard deviation. All variables exhibited a normal distribution and homogeneous variability, according to Shapiro–Wilk and Levene's tests. To compare the mean values between evaluation moments, a Student's *t* test for paired samples was performed. The analysis was performed using the Jamovi Project (Version 2.3, Sydney, Australia, 2022).

The analysis considered mean values, standard deviations, and effect sizes (Cohen's *d*). We calculated the variable mean difference divided by the pooled standard deviation to measure the effect size for mean differences between first and second evaluation, and the thresholds of 0.2, 0.3, 0.5, 0.7, and 0.9 for small, moderate, large, very large, and extremely large were used.¹⁷ The significance level was set at $p < 0.05$.

Table 2 Physiological Characteristics of the Participants

	First Evaluation (n = 13)	Second Evaluation (n = 13)	p-value	Cohen's (d)	95% CI
Maximal exercise intensity					
$\dot{V}O_{2\max}$ (L \cdot min ⁻¹)	3.70 \pm 0.740	3.46 \pm 0.592 [†]	0.003	1.0550	0.3 to 1.7
$\dot{V}O_{2\max}$ (mL \cdot min ⁻¹ \cdot kg BM ⁻¹)	56.3 \pm 5.56	52.5 \pm 4.59 [†]	< 0.001	1.2859	0.5 to 2.0
$\dot{V}O_{2\max}$ (mL \cdot min ⁻¹ \cdot kg LLLM ⁻¹)	197 \pm 14.2	198 \pm 23.0	0.083	-0.0418	-0.5 to 0.5
MAS (Km \cdot h ⁻¹)	16.69 \pm 1.65	16.15 \pm 1.28 [†]	0.047	0.6139	0.01 to 1.2
Submaximal exercise intensity – VT					
VT % $\dot{V}O_{2\max}$ (mL \cdot min ⁻¹ \cdot kg BM ⁻¹)	73.8% \pm 5.18	75.0% \pm 5.71	0.541	-0.1746	-0.7 to 0.3
VT Speed (Km \cdot h ⁻¹)	11.61 \pm 0.87	11.84 \pm 1.21	0.337	-0.2774	-0.8 to 0.2
Submaximal exercise intensity – RCP					
RCP % $\dot{V}O_{2\max}$ (mL \cdot min ⁻¹ \cdot kg BM ⁻¹)	89.1% \pm 3.28	89.5% \pm 3.27	0.776	-0.0806	-0.6 to 0.4
RCP Speed (Km \cdot h ⁻¹)	14.23 \pm 1.09	14.07 \pm 1.44	0.436	0.2234	-0.3 to 0.7

Notes: Values are expressed as mean \pm SD. Power = (1 - β); [†]p<0.05 (significant different from first evaluation).

Abbreviations: $\dot{V}O_{2\max}$, maximal oxygen uptake; BM, body mass; LLLM, lower limbs lean mass; MAS, maximum aerobic speed; VT, ventilatory threshold; RCP, respiratory compensation point; CI, confidence interval %.

Table 3 Body Composition Characteristics of Participants

	First Evaluation (n = 13)	Second Evaluation (n = 13)	p-value	Cohen's (d)	CI (95%)
BM (kg)	66.5 \pm 12.6	66.4 \pm 11.7	0.820	0.0644	-0.48 to 0.60
FM (%)	17.7 \pm 5.9	19.4 \pm 5.6	0.173	-0.4018	-0.96 to 0.17
BMI (kg/cm ²)	22.9 \pm 2.9	22.8 \pm 2.7	0.742	0.0936	-0.45 to 0.63
TLM (kg)	53.1 \pm 11.8	53.8 \pm 13.5	0.568	-0.1630	-0.70 to 0.38
LLLM (kg)	18.86 \pm 3.9	17.76 \pm 4.1 [†]	0.045	0.6199	0.12 to 1.20

Results

In the initial evaluation, participants in the present study showed significantly higher values for $\dot{V}O_{2\max}$ (L \cdot min⁻¹; $p = 0.003$, $d = 1.05$), BM-adjusted $\dot{V}O_{2\max}$ (mL \cdot min⁻¹ \cdot kg BM⁻¹; $p < 0.001$, $d = 1.2859$), and MAS (km \cdot h⁻¹; $p = 0.047$, $d = 0.6139$) than in the second evaluation. In contrast, no difference between tests was observed for $\dot{V}O_{2\max}$ adjusted for LLLM (mL \cdot min⁻¹ \cdot kg LLLM⁻¹; $p = 0.083$, $d = -0.0418$), VT % $\dot{V}O_{2\max}$ ($p = 0.541$, $d = -0.1746$), VT speed (km \cdot h⁻¹; $p = 0.337$, $d = -0.2774$), RCP % $\dot{V}O_{2\max}$ ($p = 0.776$, $d = -0.0806$), and RCP speed (km \cdot h⁻¹; $p = 0.436$, $d = 0.2234$; Table 2).

Furthermore, BM ($p = 0.820$, $d = 0.0644$), total LM (TLM; $p = 0.568$, $d = -0.1630$), and FM ($p = 0.173$, $d = -0.4018$) did not differ significantly between the two moments, with the exception of LLLM, which was lower in the second evaluation ($p = 0.045$, $d = 0.6199$; Table 3).

Discussion

The main results of the present study were as follows: (a) absolute $\dot{V}O_{2\max}$ and BM-adjusted $\dot{V}O_{2\max}$ were significantly higher in the first evaluation, (b) LLLM adjusted $\dot{V}O_{2\max}$ did not change between the two evaluations, (c) speed and $\dot{V}O_{2\max}$ percentage assessed at VT and RCP were not different between the two evaluation moments, and (d) FM (%) and TLM (kg) did not differ between the two evaluation moments. Therefore, the initial hypothesis of the study was confirmed as the different forms of $\dot{V}O_{2\max}$ expression presented different sensibilities to detect changes in training status.

The first important finding of the present study was that absolute $\dot{V}O_{2\max}$ and BM-adjusted $\dot{V}O_{2\max}$ were significantly higher in the first evaluation than in the second. Therefore, these two variables were sensitive enough to detect different levels of training. It is important to note that the second evaluation was performed 1 month after the target race (at the end of September or beginning of October), when the training volumes and intensities decreased

significantly. Therefore, these results are expected, because it is reasonable to assume that participants were in a detraining period.

Traditionally, $\dot{V}O_{2\max}$ has been defined as the highest rate at which oxygen can be taken in by the lungs, transported by the blood, and used by the muscles during whole-body activity, such as cycling or running.⁵ Even though various physiological factors, such as pulmonary diffusing capacity, ventilatory capacity, maximal cardiac output, blood oxygen carrying capacity, and skeletal muscle capacity to consume oxygen, can limit $\dot{V}O_{2\max}$, there are several reasons to believe that the most important limiting factors are those associated with maximal cardiac output.^{18,19} Pulmonary variables were excluded because the average individual does not have arterial oxygen desaturation even at maximal exercise intensity.^{18,19} Furthermore, the possibility of skeletal muscle limitation as a restricting factor for $\dot{V}O_{2\max}$ was dismissed. This is because enhancing the oxygen supply to the active muscle mass, for instance, through higher inspired oxygen levels or blood doping, increases $\dot{V}O_{2\max}$.

As a result, the observed changes relative to the absolute and BM-adjusted $\dot{V}O_{2\max}$ represent a lower central capacity to maintain the previous maximal cardiac output. This sensitivity of absolute and BM-adjusted $\dot{V}O_{2\max}$ in identifying training differences is consistent with previous literature findings. It has previously been proven that increasing training has a positive effect, with overall aerobic capacity increasing by approximately $0.4 \pm 0.2 \text{ L} \cdot \text{min}^{-1}$ after a training period.²⁰ These findings support using absolute and BM-adjusted $\dot{V}O_{2\max}$ values to demonstrate training status change.

Similarly, the MAS was also lower in the second evaluation than in the first, and one more time, it is important to remember that the second evaluation was performed 1 month after the target race, and when the training volumes and intensities have dropped. MAS is known to directly correlate with $\dot{V}O_{2\max}$ and is an important predictor of an athlete's performance, which performs even better than $\dot{V}O_{2\max}$ in some race distances, such as Olympic distance.² The reduction in absolute and BM-adjusted $\dot{V}O_{2\max}$ during the second evaluation might be attributed to the decrease in MAS.

However, in addition to physiological differences, the results may also have been influenced by the fact that both sexes were analyzed together, and it has been previously demonstrated that the effects of resistance training on muscular function, and the effects of endurance training on $\dot{V}O_{2\max}$ were larger in males compared with female sex.^{21,22} In the same direction, the age varied considerably for the participants included in the present study and it is very likely this factor can contribute to training response, despite more studies should be done to verify this hypothesis.²³

Regarding, LLLM-adjusted $\dot{V}O_{2\max}$ did not change during the same follow-up period. As shown in the present results, it has previously been demonstrated that there is no significant difference in LM-adjusted $\dot{V}O_{2\max}$ in a 1-yr training cycle for endurance or sprint athletes³. The lower limb lean mass-adjusted $\dot{V}O_{2\max}$ has been claimed to reflect the ability of the muscle mass to consume oxygen and is used as an index of "aerobic muscle quality", referring to the amount of oxygen consumed per kilogram of muscle mass.^{3,7}

Despite changes in training status, aerobic muscle quality did not change over the present study's follow-up period. However, this discovery should be interpreted alongside the conclusion that while this variable signifies oxygen extraction by muscle mass, it might not represent the utmost extraction capacity but rather the extraction evaluated during maximum exercise. There is a wide range of reported values for LM-adjusted $\dot{V}O_{2\max}$, ranging from less than 100 to more than $700 \text{ mL} \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$.^{24–27} The amount of muscle mass engaged in physical activity is one of the main reasons for this wide variation.²⁸ Physical activities involving small muscle mass provide greater LM-adjusted $\dot{V}O_{2\max}$ than full body dynamic exercises. This is because, as previously stated, the absolute $\dot{V}O_{2\max}$ values are limited in most participants by cardiac output rather than skeletal muscle oxygen extraction capacity. Therefore, if a small muscle mass performs an activity, oxygen delivery to the active muscles can increase oxygen muscular extraction and, consequently, the LM-adjusted $\dot{V}O_{2\max}$ values. However, this variable has received far less attention than the absolute or BM-adjusted $\dot{V}O_{2\max}$, and more studies presenting accurate, reliable, and precise methods to estimate muscle mass and oxygen uptake should be conducted to confirm this hypothesis.

Despite the significant decrease in $\dot{V}O_{2\max}$ values, there was no significant difference in the ventilatory thresholds. Similarly, there was no difference between the two evaluations in the speed observed at ventilatory thresholds.²⁹ Ventilatory thresholds are mainly influenced by peripheral adaptations to training stimuli, with little contribution from central aspects.^{5,30} Furthermore, the operating economy affects the ventilatory thresholds.³¹ Therefore, these results support the hypothesis that research participants could sustain peripheral adaptations to training stimulus despite a decrease in absolute and BM-adjusted $\dot{V}O_{2\max}$.

As shown in Table 3, there were no statistically significant differences in BM, TLM (kg), or FM (%) between the two evaluation time points. Anthropometric data (ie, body fat, abdominal circumference, BM, body mass index, and LM) are well-known to be associated with performance in endurance athletes of both sexes.^{32,33} BM was associated with the final race time³⁴ and marathon split³⁵ in male triathletes competing over long distances (Ironman).

It has been demonstrated that among Olympic-distance amateur triathletes, LM for female athletes and FM for male athletes are important variables that comprise predictive performance regression equations.³³ Therefore, the nonsignificant differences observed between the two moments for anthropometric variables indicate that these variables have a minimal impact on the other determinants of performance evaluated throughout the season, which changed significantly, such as absolute, BM-adjusted $\dot{V}O_2\text{max}$ and MAS, indicating that there are independent factors associated with performance.

Limitations

The volume and intensity of training were not managed, which can be considered a bias of the study. However, it is important to note that all the athletes were preparing for long-distance races, such as the Ironman (3.8 km swimming, 180 km cycling, and 42 km running) or the 70.3 Ironman (1.9 km swimming, 90 km cycling, and 21 km running) by the same coach using the same training methodology, which may mitigate this possible bias. Second, the large range of age among participants from both sexes can also be a bias of the study. Third, caution should be taken in interpreting the results of no significant difference between means, once the sample size evaluated in the present study was not large. Therefore, to clarify this issue, the authors suggest that future researchers should be performed considering male and female athletes separately and studying a larger sample size with a smaller range of age.

Conversely, a strength of the study is that all athletes took both assessments in the same phase of training, the first evaluation during the pre-season and the second evaluation approximately 30 days after the year's target competition.

Conclusion

In conclusion, absolute or total body mass adjusted $\dot{V}O_2\text{max}$ are sensitive measures to identify physical training status changes among amateur triathletes. However, lower limb lean mass adjusted $\dot{V}O_2\text{max}$ was not able to identify changes in physical training status. Therefore, coaches and athletes aiming to verify the endurance training effects on $\dot{V}O_2\text{max}$ should prefer to look for absolute or total body mass adjusted $\dot{V}O_2\text{max}$ instead of lower limb lean mass adjusted $\dot{V}O_2\text{max}$.

Data Sharing Statement

Data supporting the reported results can be requested from the corresponding author.

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of UNIFESP (protocol code 5.059.538).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising, or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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