

ORIGINAL PAPER **OPEN ACCESS**

# Ventilatory Thresholds Differences According to Aerobic Fitness Level in 1450 Males and 241 Females on Cycle-Ergometer: A Cross-Sectional Study

José Antonio Benítez-Muñoz<sup>1</sup>  | María Alcocer-Ayuga<sup>1,2</sup>  | Rocío Cupeiro<sup>1</sup>  | Isabel Guisado-Cuadrado<sup>1</sup>  | Miguel Ángel Rojo-Tirado<sup>1</sup>  | Víctor M. Alfaro-Magallanes<sup>1,3,4</sup>  | Nuria Romero-Parra<sup>1,5</sup>  | Eliane Aparecida-Castro<sup>1,6,7</sup>  | Domingo J. Ramos-Campo<sup>1</sup>  | Alberto Armero-Sotillo<sup>1</sup> | Ana Belén Peinado<sup>1</sup>  | Pedro J. Benito<sup>1</sup> 

<sup>1</sup>LFE Research Group, Department of Health and Human Performance, Faculty of Physical Activity and Sport Science (INEF), Universidad Politécnica de Madrid, Madrid, Spain | <sup>2</sup>Olympia Sport & Lifestyle Center, Grupo Quironsalud, Madrid, Spain | <sup>3</sup>Department of Health Sciences, Institute for Sustainability & Food Chain Innovation, Public University of Navarre, Pamplona, Spain | <sup>4</sup>Navarra Institute for Health Research (IdiSNA), Pamplona, Spain | <sup>5</sup>Department of Physical Therapy, Occupational Therapy, Rehabilitation and Physical Medicine, Faculty of Health Sciences, Universidad Rey Juan Carlos, Madrid, Spain | <sup>6</sup>Graduate Programme in Human Development and Technology, São Paulo State University (UNESP), Rio Claro, Brazil | <sup>7</sup>Department of Physical Education, School of Sciences (FC), São Paulo State University (UNESP), Bauru, Brazil

**Correspondence:** José Antonio Benítez-Muñoz ([joseantonio.benitez.munoz@upm.es](mailto:joseantonio.benitez.munoz@upm.es)) | Pedro J. Benito ([pedroj.benito@upm.es](mailto:pedroj.benito@upm.es))

**Received:** 2 January 2025 | **Revised:** 12 May 2025 | **Accepted:** 15 May 2025

**Funding:** J.A.B.M. and I.G.C. were supported by a grant provided by Universidad Politécnica de Madrid. V.M.A.M. was supported by a Margarita Salas grant funded by Ministerio de Ciencia e Innovación and by the European Union NextGenerationEU/RRF—Technical University of Madrid.

**Keywords:** heart rate | oxygen uptake | power output | VT1 | VT2

## ABSTRACT

The aim of the study was to analyze the influence of an aerobic fitness level on the percentage of maximum oxygen consumption, heart rate, and power output ( $\%VO_{2max}$ ,  $\%HR_{max}$ , and  $\%W_{max}$ ) at which ventilatory thresholds 1 (VT1) and 2 (VT2) occur during a ramp incremental cycle-ergometer test in males and females considering age. 1450 males and 241 females performed a ramp incremental exercise test until exhaustion to determine VT1, VT2, and  $VO_{2max}$ . Combining the oxygen consumption at VT1, VT2, and  $VO_{2max}$  by clustering analysis, males were classified as a low, medium, or high aerobic fitness level and females were classified as a low or high aerobic fitness level. Results showed  $VO_{2max}$  was very poorly correlated with the  $\%VO_{2max}$  at which VT1 and VT2 occur ( $r \leq 0.115$ ), whereas oxygen consumption at VT1 and VT2 showed a stronger positive association with the  $\%VO_{2max}$  at which VT1 and VT2 occur, respectively ( $r = 0.357$ – $0.604$ ). Furthermore, the  $\%VO_{2max}$  at which VT1 and VT2 occur were greater the higher the aerobic fitness level (all  $p \leq 0.002$ ), observing a high heterogeneity in the  $\%VO_{2max}$  at which VT1 and VT2 occur even stratifying the sample by sex and aerobic fitness levels. In conclusion, the percentage of maximum at which VT1 and VT2 occur are better related to oxygen consumption at VT1 and VT2, respectively, than to  $VO_{2max}$ . Moreover, the common strategy consisting of establishing exercise intensity as a fixed percentage of maximum might not be effective to match intensity across individuals even if sex and aerobic fitness levels is considered.

**Trial Registration:** NCT06246760.

## Summary

- The percentage of maximum at which VT1 and VT2 occur are more strongly related to oxygen consumption at VT1 and VT2, respectively, than to  $VO_{2max}$ .
- When the aerobic fitness level is determined by combining oxygen consumption at VT1 and VT2 alongside  $VO_{2max}$ , VT1 and VT2 occur at a higher percentage of maximum as the aerobic fitness level increases. Whereas, if only  $VO_{2max}$  is considered (as traditionally done), no effect of the aerobic fitness level on the position of thresholds was found.
- Despite stratifying participants by sex and aerobic fitness levels, it is observed that a wide heterogeneity in the percentage of maximum at which thresholds occur. This indicates that the same percentage of maximum could produce different physiological responses despite considering aerobic fitness levels and sex.

## 1 | Introduction

Traditionally, exercise intensity is prescribed as a fixed percentage of maximal oxygen consumption ( $\%VO_{2max}$ ), heart rate ( $\%HR_{max}$ ), or heart rate reserve, velocity ( $\%V_{max}$ ), or power output ( $\%W_{max}$ ). Expressing these variables as a percentage of their maximum values helps standardize intensity across individuals (Mann et al. 2013). It is widely assumed that exercising at the same percentage of maximum exerts similar physiological stress across individuals, an approach commonly used in recent studies (Coates et al. 2023; Sevilla-Lorente et al. 2024) and general exercise guidelines (Garber et al. 2011; Haskell et al. 2007). However, this approach has been questioned in excellent studies (Iannetta et al. 2020; Jamnick et al. 2020; S. Meyler et al. 2021) because along the intensity spectrum it is possible to identify distinct intensity zones—moderate, heavy, and severe—which elicit widely different physiological responses (Iannetta et al. 2022). In an incremental test, the transition from moderate to heavy intensity is often marked by the first ventilatory threshold (VT1) and the shift from heavy to severe intensity is indicated by the second ventilatory threshold (VT2) (Keir et al. 2022). The percentage of maximum at which these thresholds occur varies widely among individuals, regardless of whether  $\%VO_{2max}$ ,  $\%HR_{max}$ , or  $\%W_{max}$  is used (Iannetta et al. 2020). In this sense, prescribing exercise intensity based on physiological thresholds rather than fixed percentages of maximum reduces heterogeneity in acute exercise response (S. Meyler et al. 2023) and elicits superior mean changes in  $VO_{2max}$  and increased the likelihood of increasing  $VO_{2max}$  (Byrd et al. 2019; S. J. R. Meyler et al. 2025; Preobrazenski et al. 2019; Weatherwax et al. 2019; Wolpern et al. 2015).

In the literature, there is considerable controversy regarding the  $\%VO_{2max}$  at which thresholds occur across individuals with different aerobic fitness levels. Although some suggest that these thresholds appear at similar percentages of  $VO_{2max}$  regardless of the aerobic fitness level (Iannetta et al. 2020; Støa et al. 2020), others have found different  $\%VO_{2max}$  values at thresholds across individuals with the diverse aerobic fitness level (Alejo et al. 2022; Benítez-Muñoz et al. 2024; Hetlelid et al. 2015; Zapico et al. 2007). One of the main factors that may

be contributing to these discrepancies in the literature is the way used to classify participants' aerobic fitness level. In this regard, oxygen consumption at thresholds has been shown to be highly relevant for both health and performance, potentially even surpassing the relevance of  $VO_{2max}$  (for further review see (Poole et al. 2021)). Hence, it would be advisable to assess aerobic fitness using all three primary physiological markers (VT1, VT2, and  $VO_{2max}$ ) to obtain a more comprehensive understanding of participants' aerobic fitness status. This novel approach to classifying aerobic fitness levels could help clarify whether the aerobic fitness level influences the  $\%VO_{2max}$  at which VT1 and VT2 occur ( $\%VO_{2max}VT1$  and  $\%VO_{2max}VT2$ , respectively) across individuals with different aerobic fitness levels in cycle-ergometer, ideally through a study with a large sample size. Additionally, there is a shortage of research investigating differences in the  $\%HR_{max}$  and  $\%W_{max}$  at which thresholds occur based on the aerobic fitness level. If the aerobic fitness level does affect the  $\%VO_{2max}$ ,  $\%HR_{max}$ , or  $\%W_{max}$  at which thresholds are reached, standardizing intensity as a fixed percentage of maximum may produce additional differences in the physiological responses when considering individuals with different levels of aerobic fitness. Moreover, reporting the reference standard values of these variables based on the aerobic fitness level and sex on cycle-ergometer would greatly enhance the accuracy at interpreting an individual's aerobic fitness level.

Thus, the aim of the present study was to analyze the influence of the aerobic fitness level on the  $\%VO_{2max}$ ,  $\%HR_{max}$ , or  $\%W_{max}$  at which VT1 and VT2 occur, separately for males and females, while also considering age, during a ramp incremental cycle-ergometer test. Additionally, we offer reference values for these same variables and additional variables according to the aerobic fitness level, leveraging the large sample size.

## 2 | Materials and Methods

### 2.1 | Participants

This study involved 1450 males ( $73.3 \pm 10.48$  [30–125] kg,  $175.2 \pm 6.96$  [136–200] cm, and  $33.2 \pm 12.74$  [9–83] years) and 241 females ( $59.3 \pm 10.41$  [30–104] kg,  $162.7 \pm 7.06$  [136–183] cm, and  $29.9 \pm 13.9$  [9–69] years) who voluntarily visited our laboratory to undergo an exercise test. Data were collected between the years 2000 and 2024. Participants were instructed to abstain from vigorous exercise for 2 days prior to the test and to eat at least 2 hours before the test. All participants received detailed information about the potential risks and benefits of the study and provided written informed consent to participate. The protocols and procedures were approved by the Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association Declaration of Helsinki (1964) and subsequent amendments. This study was approved by the Human Research Ethics Committee of the University where the experiments were carried out. Data and information obtained in the project were treated as confidential in accordance with the current national legislation governing personal data protection (Organic Law 3/2018). Therefore, access to the database was restricted to researchers involved in the study. Clinical trial registration: NCT06246760.

## 2.2 | Ramp Incremental Exercise Test

The ramp incremental exercise test was performed on a computerized cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). The ergometer was fitted to each participant. The incremental tests were tailored to each participant's training experience (sporting background) to accurately determine VT1, VT2, and  $VO_{2max}$ . Two primary considerations guided the individualization process: first, to avoid excessively long protocols (> 26 min) for well-trained individuals, which could hinder reaching  $VO_{2max}$  due to peripheral fatigue (Midgley et al. 2008); and second, to prevent overly demanding protocols for poorly trained individuals, which could impede the proper determination of VT1 and VT2 due to the protocol duration being too short (Bentley et al. 2007). The test commenced with a 2–10 min warm-up at a constant intensity between 20 and 100 W. Subsequently, the incremental phase involved a ramp test starting from 20 to 110 W, with the power output increasing by 12–25 W/min. The incremental phase concluded when the participant reached maximum exhaustion, which was defined as the inability to maintain a cadence above 70 rpm for longer than ~5 s (Benítez-Muñoz et al. 2025). Finally, a three-minute active recovery at the same intensity as the warm-up was followed by a two-minute passive recovery period. The cadence had to be kept stable between 70 and 90 rpm. Participants had to be seated during the whole test. Throughout the test, breath-by-breath oxygen consumption and beat-by-beat heart rate (HR) were analyzed using a Jaeger Oxycon Pro (Erich Jaeger, Viasys Healthcare, Friedberg, Germany) and JAEGER Vyntus CPX (Jaeger-CareFusion, Hoechst, Germany) previously calibrated according to the manufacturer's specifications.

## 2.3 | Data Extracted From Incremental Tests

VT1 and VT2 were determined as previously published (Rabadán et al. 2011). In brief, VT1 was identified using: (a) the V-slope method, where VT1 represents the breakpoint of the  $VCO_2/VO_2$  relationship; (b) the first exponential increment in ventilation (VE) relative to time; and (c) the first increase in the VE/ $VO_2$  relationship without accompanying increases in the VE/ $VCO_2$  relationship relative to time. VT2 was determined: (a) as the second exponential increment in ventilation relative to time; (b) as the intensity corresponding to the second increase in the VE/ $VO_2$  relationship alongside a concurrent rise in the VE/ $VCO_2$  relationship relative to time; and (c) as a concomitant rise in  $PETO_2$  and fall in  $PETCO_2$  relative to time. Once the time at which VT1 and VT2 occurred was identified, the values of the different variables obtained at that time were taken.  $VO_{2max}$  was established as the highest value averaged every 15 s. The test was considered maximal if three of the following four criteria were met: voluntary exhaustion of the participant,  $HR \geq 95\%$  of the theoretical  $HR_{max}$  calculated as 220 minus the age,  $RER \geq 1.10$ , and a plateau in  $VO_2$  despite an increase in exercise intensity. All tests were independently evaluated by two researchers, with a third researcher included in cases of disagreement. The following variables were determined: oxygen consumption values at VT1 ( $VO_{2VT1}$ ), VT2 ( $VO_{2VT2}$ ), and maximum ( $VO_{2max}$ ) relative to body weight; percentage of maximal oxygen consumption at VT1 ( $\%VO_{2maxVT1}$ ) and VT2 ( $\%VO_{2maxVT2}$ ); an absolute heart rate at VT1 ( $HR_{VT1}$ ), VT2 ( $HR_{VT2}$ ), and maximum ( $HR_{max}$ ); percentage

of the maximal heart rate at VT1 ( $\%HR_{maxVT1}$ ) and VT2 ( $\%HR_{maxVT2}$ ); power output at VT1 ( $W_{VT1}$ ), VT2 ( $W_{VT2}$ ), and maximum ( $W_{max}$ ); and percentage of maximal power output at VT1 ( $\%W_{maxVT1}$ ) and VT2 ( $\%W_{maxVT2}$ ).

## 2.4 | Aerobic Fitness Level Classification

Oxygen consumption at each threshold and maximum was chosen to determine the aerobic fitness level for two main reasons: (a) its significant impact on health and performance (Poole et al. 2021) and (b) because the exercise protocol appears not to influence  $VO_{2VT1}$ ,  $VO_{2VT2}$ , and  $VO_{2max}$  (Iannetta et al. 2019; Leo et al. 2017; Scheuermann and Kowalchuk 1998), provided the main considerations outlined in the ramp incremental exercise test section are met. A two-step cluster analysis was conducted to determine the optimal number of clusters based on three quantitative variables ( $VO_{2VT1}$ ,  $VO_{2VT2}$ , and  $VO_{2max}$ ), using the Bayesian Information Criteria (BIC) to identify the clusters with the lowest BIC. Additionally, the Silhouette index was used to assess clustering performance, categorized as poor (less than 0.2), fair (0.2–0.5), or good (> 0.5) (Wendler and Gröttrup 2021). The predictor importance of a variable for cluster formation ranged from 0 to 1, derived from a two-step algorithm. Consequently, males were classified into low, medium, or high aerobic fitness level categories; whereas females were classified into low or high aerobic fitness level categories (as shown below).

## 2.5 | Statistical Analysis

All the data are presented as the means  $\pm$  standard deviation (SD) (minimum–maximum). The data were tested for normal distribution with the Kolmogorov–Smirnov test and for homogeneity of variances with Levene's test. All analyses were performed in males and females independently. Pearson correlation coefficients were calculated to assess the association between  $VO_{2max}$  and the  $\%VO_{2max}$ ,  $\%HR_{max}$ , and  $\%W_{max}$  at which VT1 and VT2 occur; between  $VO_{2VT1}$  and the  $\%VO_{2max}$ ,  $\%HR_{max}$  and  $\%W_{max}$  at which VT1 occur; and between  $VO_{2VT2}$  and the  $\%VO_{2max}$ ,  $\%HR_{max}$  and  $\%W_{max}$  at which VT2 occur. ANCOVA (age as a covariable) was used to study the effect of aerobic fitness level on the different variables determined at VT1 ( $VO_{2VT1}$ ,  $\%VO_{2maxVT1}$ ,  $HR_{VT1}$ ,  $\%HR_{maxVT1}$ ,  $W_{VT1}$ , and  $\%W_{maxVT1}$ ), at VT2 ( $VO_{2VT2}$ ,  $\%VO_{2maxVT2}$ ,  $HR_{VT2}$ ,  $\%HR_{maxVT2}$ ,  $W_{VT2}$ , and  $\%W_{maxVT2}$ ), and at maximum ( $VO_{2max}$ ,  $HR_{max}$ , and  $W_{max}$ ). Bonferroni post hoc tests were conducted where significant main effects were found in any of the analyzed factors. The effect size of the ANCOVA was calculated using partial eta-squared ( $\eta^2$ ) and the small, moderate, and large effects corresponded to values equal or greater than 0.001, 0.059, and 0.138, respectively (Cohen 1988). The criteria to interpret the strength of the r coefficients were as follows: trivial (< 0.1), small (0.1–0.3), moderate (0.3–0.5), high (0.5–0.7), very high (0.7–0.9), or practically perfect (> 0.9) (Hopkins et al. 2009). Statistical analyses were carried out with the statistical software jamovi V1.6 (jamovi, Sydney, Australia) and SPSS software 29 version (IBM Corp., Armonk, NY, USA). The significance level was set at  $p < 0.05$ .

### 3 | Results

#### 3.1 | Correlations

In males,  $VO_{2max}$  was poorly correlated with VT1 position when expressed as  $\%VO_{2max}$  ( $r = 0.091$  and  $p < 0.001$ ),  $HR_{max}$  ( $r = 0.162$  and  $p < 0.001$ ), or  $\%W_{max}$  ( $r = 0.342$  and  $p = 0.005$ ) or with VT2 position when expressed as  $\%VO_{2max}$  ( $r = 0.115$  and  $p < 0.001$ ),  $HR_{max}$  ( $r = 0.092$  and  $p < 0.001$ ), or  $\%W_{max}$  ( $r = 0.166$  and  $p = 0.003$ ). Similar results were found in females, finding no or very low correlations between  $VO_{2max}$  with VT1 position when expressed as  $\%VO_{2max}$  ( $r = 0.027$  and  $p = 0.68$ ),  $HR_{max}$  ( $r = 0.214$  and  $p = 0.001$ ), or  $\%W_{max}$  ( $r = 0.274$  and  $p = 0.002$ ) or with VT2 position when expressed as  $\%VO_{2max}$  ( $r = 0.053$  and  $p = 0.423$ ),  $HR_{max}$  ( $r = -0.036$  and  $p = 0.594$ ), or  $\%W_{max}$  ( $r = 0.265$  and  $p = 0.003$ ).

In contrast, a moderate correlation was found between  $VO_{2VT1}$  with  $\%VO_{2max}VT1$  and between  $VO_{2VT2}$  with  $\%VO_{2max}VT2$  in both sexes. Additionally,  $VO_{2VT1}$  was moderately correlated with  $\%HR_{max}VT1$  and  $\%W_{max}VT1$  in both sexes and  $VO_{2VT2}$  was moderately correlated with  $\%HR_{max}VT2$ , only in males, and  $\%W_{max}VT2$  in both sexes (Figure 1).

#### 3.2 | Cluster Analysis

Using the two-step cluster analysis, the optimal number of clusters identified was three for males, categorized as a low, medium, and high aerobic fitness level, and two for females, categorized as a low and high aerobic fitness level. The Silhouette index for the models was 0.6 in both sexes, indicating good clustering quality. In males,  $VO_{2max}$ ,  $VO_{2VT1}$ , and  $VO_{2VT2}$  had the same importance value (1). In females,  $VO_{2VT2}$  was the most critical variable (1), followed by  $VO_{2max}$  (0.95) and  $VO_{2VT1}$  (0.79).

#### 3.3 | Aerobic Fitness Level

$\%VO_{2max}VT1$  and  $\%VO_{2max}VT2$  were different according to the aerobic fitness level, observing higher values as the aerobic fitness level increased in both sexes.  $VO_{2VT1}$ ,  $VO_{2VT2}$ , and  $VO_{2max}$  were higher the higher the aerobic fitness level of the participant in both sexes (Table 1).

$\%HR_{max}VT1$  was higher with an increasing aerobic fitness level, and  $\%HR_{max}VT2$  was higher in a medium and high aerobic fitness level compared to low in males. In females,  $\%HR_{max}VT1$  was higher in a high aerobic fitness level compared to low, whereas  $\%HR_{max}VT2$  remained similar across the aerobic fitness level.  $HR_{VT1}$  and  $HR_{VT2}$  increased as the aerobic fitness level increased in both sexes.  $HR_{max}$  was higher in a high aerobic fitness level compared to medium and low in males, and it was higher in high compared to low in females (Table 2).

$\%W_{max}VT1$  was higher as the aerobic fitness level increased in both sexes.  $\%W_{max}VT2$  was higher in a medium and high aerobic fitness level compared to low in males, and it was higher in high compared to low in females.  $W_{VT1}$ ,  $W_{VT2}$ , and  $W_{max}$  were higher the higher the aerobic fitness level (Table 3).

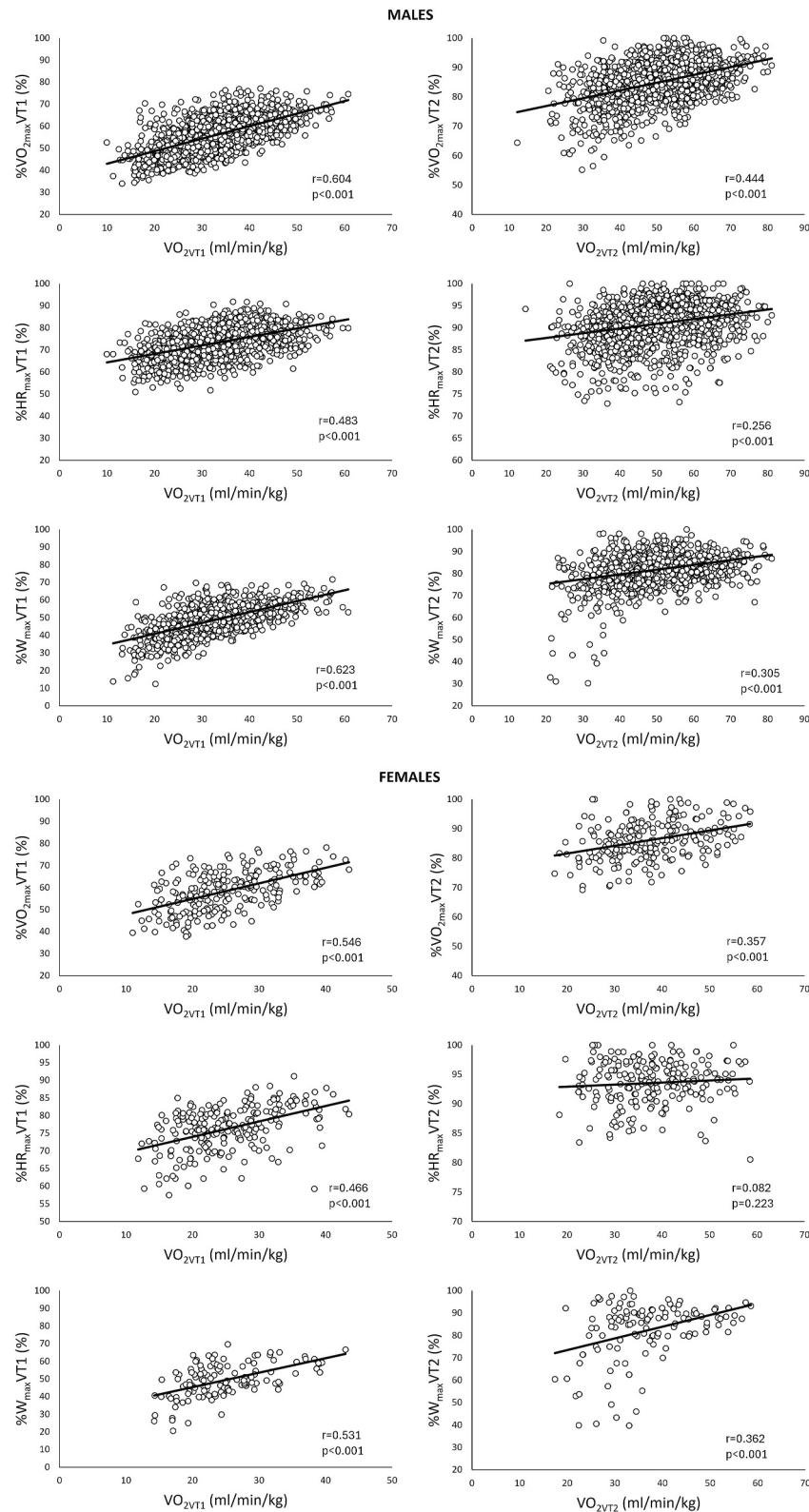
Finally, it is observed a wide heterogeneity in the VT1 and VT2 position when expressed as  $\%VO_{2max}$ ,  $\%HR_{max}$ , or  $\%W_{max}$  (Figure 2).

### 4 | Discussion

The key findings of this study revealed that  $\%VO_{2max}VT1$  and  $\%VO_{2max}VT2$  are more closely associated with  $VO_{2VT1}$  and  $VO_{2VT2}$ , respectively, rather than with  $VO_{2max}$ . Moreover, the  $\%VO_{2max}$ ,  $\%HR_{max}$ , and  $\%W_{max}$  at which thresholds occur increased the higher the aerobic fitness level as determined using our novel classification approach that integrates  $VO_{2VT1}$ ,  $VO_{2VT2}$ , and  $VO_{2max}$ . Even stratifying participants by sex and aerobic fitness levels, considerable interindividual variability in the percentage of maximum at which VT1 and VT2 occurred persisted. These results suggest that considering only  $VO_{2max}$  masks a fitness-specific change, such as the  $\%VO_{2max}$ , at which VT1 and VT2 occur. Moreover, the conventional approach of setting exercise intensity as a fixed percentage of maximum may not effectively standardize intensity despite considering aerobic fitness levels and sex. Additionally, the data from this study can serve as standard reference values for these variables in relation to the aerobic fitness level, making it easier to interpret an individual's aerobic fitness level.

The novelty of this work lies in its approach to classifying individuals, which is essential for identifying the impact of aerobic fitness level on threshold positions by examining oxygen consumption at the three primary physiological markers (VT1, VT2, and  $VO_{2max}$ ). In contrast, focusing solely on  $VO_{2max}$ , as traditionally done, does not reveal any effect of the aerobic fitness level on these thresholds. This finding underscores that relying exclusively on  $VO_{2max}$  overlooks the influence of aerobic fitness level on submaximal metrics, such as  $\%VO_{2max}VT1$  and  $\%VO_{2max}VT2$ , suggesting that  $VO_{2max}$  alone may not effectively capture key submaximal variables. Moreover, assessing  $VO_{2VT1}$ ,  $VO_{2VT2}$ , and  $VO_{2max}$ , rather than only  $VO_{2max}$ , is highly relevant, as oxygen consumption at thresholds has demonstrated substantial importance for both health and performance, potentially surpassing the relevance of  $VO_{2max}$  (Poole et al. 2021). Consequently, we classified participants by combining  $VO_{2VT1}$ ,  $VO_{2VT2}$ , and  $VO_{2max}$  in our cluster analysis. This new classification establishes reference values for the reported variables according to the aerobic fitness level. Therefore, the values of an individual performing a maximal incremental test can be compared to the values reported in our study, as long as both the characteristics of the evaluated individual and the maximal incremental test are very similar to the average characteristics of the present study. This comparison may allow to classify the individual into one of the aerobic fitness level groups defined in our sample. In the case of males, three levels are available (low, medium, and high), whereas in females, two levels (low and high) were identified.

$VO_{2VT1}$  and  $VO_{2VT2}$  exert a positive and stronger association on  $\%VO_{2max}VT1$  and  $\%VO_{2max}VT2$ , respectively, compared to  $VO_{2max}$ , which presented a very poor association. This indicates the  $\%VO_{2max}VT1$  and  $\%VO_{2max}VT2$  are mainly associated with  $VO_{2VT1}$  and  $VO_{2VT2}$ , respectively, rather than  $VO_{2max}$ . It seems



**FIGURE 1** | Correlation between  $VO_{2VT1}$  with % $VO_{2max}VT1$ , % $HR_{max}VT1$ , and % $W_{max}VT1$  and between  $VO_{2VT2}$  with % $VO_{2max}VT2$ , % $HR_{max}VT2$ , and % $W_{max}VT2$ .  $VO_{2VT1}$  and  $VO_{2VT2}$ : relative to body weight values of oxygen consumption at ventilatory thresholds 1 and 2, respectively; % $VO_{2max}VT1$  and % $VO_{2max}VT2$ : percentage of maximal oxygen consumption at ventilatory thresholds 1 and 2, respectively; % $HR_{max}VT1$  and % $HR_{max}VT2$ : percentage of the maximal heart rate at ventilatory thresholds 1 and 2, respectively; % $W_{max}VT1$  and % $W_{max}VT2$  percentage of maximal power output at ventilatory thresholds 1 and 2, respectively.

**TABLE 1** | Differences according to the aerobic fitness level on the different variables related to oxygen consumption.

|                                | Aerobic fitness level | N   | Mean | ± | SD                  | Aerobic fitness level effect                            |
|--------------------------------|-----------------------|-----|------|---|---------------------|---------------------------------------------------------|
| Males                          |                       |     |      |   |                     |                                                         |
| VO <sub>2VT1</sub> (mL/min/kg) | Low                   | 316 | 22.3 | ± | 3.81                | $F(2,1434) = 1313.26$ $p < 0.001$<br>$\eta^2 p = 0.647$ |
|                                | Medium                | 699 | 31.5 | ± | 4.11 <sup>a</sup>   |                                                         |
|                                | High                  | 424 | 42.1 | ± | 5.65 <sup>a,b</sup> |                                                         |
| VO <sub>2VT2</sub> (mL/min/kg) | Low                   | 316 | 34.9 | ± | 5.09                | $F(2,1434) = 1989.3$ $p < 0.001$<br>$\eta^2 p = 0.735$  |
|                                | Medium                | 699 | 47.6 | ± | 4.68 <sup>a</sup>   |                                                         |
|                                | High                  | 424 | 62.7 | ± | 5.77 <sup>a,b</sup> |                                                         |
| VO <sub>2max</sub> (mL/min/kg) | Low                   | 316 | 43.3 | ± | 5.78                | $F(2,1434) = 1819$ $p < 0.001$<br>$\eta^2 p = 0.717$    |
|                                | Medium                | 699 | 56.2 | ± | 5.08 <sup>a</sup>   |                                                         |
|                                | High                  | 424 | 72.4 | ± | 6.12 <sup>a,b</sup> |                                                         |
| %VO <sub>2max</sub> VT1 (%)    | Low                   | 316 | 51.7 | ± | 7.78                | $F(2,1429) = 94.3$ $p < 0.001$<br>$\eta^2 p = 0.117$    |
|                                | Medium                | 696 | 56.2 | ± | 7.61 <sup>a</sup>   |                                                         |
|                                | High                  | 422 | 58.1 | ± | 7.34 <sup>a,b</sup> |                                                         |
| %VO <sub>2max</sub> VT2 (%)    | Low                   | 313 | 81.2 | ± | 6.99                | $F(2,1430) = 94.8$ $p < 0.001$<br>$\eta^2 p = 0.117$    |
|                                | Medium                | 698 | 85   | ± | 6.48 <sup>a</sup>   |                                                         |
|                                | High                  | 424 | 86.6 | ± | 5.61 <sup>a,b</sup> |                                                         |
| Females                        |                       |     |      |   |                     |                                                         |
| VO <sub>2VT1</sub> (mL/min/kg) | Low                   | 107 | 19.9 | ± | 3.3                 | $F(1,223) = 260.42$ $p < 0.001$<br>$\eta^2 p = 0.539$   |
|                                | High                  | 120 | 30.1 | ± | 5.4 <sup>a</sup>    |                                                         |
| VO <sub>2VT2</sub> (mL/min/kg) | Low                   | 107 | 29.7 | ± | 4.7                 | $F(1,223) = 375.86$ $p < 0.001$<br>$\eta^2 p = 0.628$   |
|                                | High                  | 120 | 43.9 | ± | 5.7 <sup>a</sup>    |                                                         |
| VO <sub>2max</sub> (mL/min/kg) | Low                   | 107 | 35.3 | ± | 5.2                 | $F(1,223) = 342$ $p < 0.001$<br>$\eta^2 p = 0.605$      |
|                                | High                  | 120 | 50.4 | ± | 6.3 <sup>a</sup>    |                                                         |
| %VO <sub>2max</sub> VT1 (%)    | Low                   | 107 | 56.8 | ± | 8.4                 | $F(1,223) = 9.57$ $p = 0.002$<br>$\eta^2 p = 0.041$     |
|                                | High                  | 120 | 59.9 | ± | 9.1 <sup>a</sup>    |                                                         |
| %VO <sub>2max</sub> VT2 (%)    | Low                   | 105 | 84.7 | ± | 6.8                 | $F(1,221) = 12.49$ $p < 0.001$<br>$\eta^2 p = 0.054$    |
|                                | High                  | 120 | 87.2 | ± | 6.1 <sup>a</sup>    |                                                         |

Note: VT1 and VT2: ventilatory thresholds 1 and 2, respectively; VO<sub>2VT1</sub>, VO<sub>2VT2</sub>, and VO<sub>2max</sub>: relative to body weight values of oxygen consumption at VT1, VT2, and maximum, respectively; %VO<sub>2max</sub>VT1 and %VO<sub>2max</sub>VT2: percentage of maximal oxygen consumption at VT1 and VT2, respectively.

<sup>a</sup>Significant difference from a low aerobic fitness level.

<sup>b</sup>Significant difference from a medium aerobic fitness level.

that individuals with higher VO<sub>2max</sub> do not necessarily have a VO<sub>2VT1</sub> and VO<sub>2VT2</sub> closer to VO<sub>2max</sub> (i.e., greater %VO<sub>2max</sub>VT1 and %VO<sub>2max</sub>VT2). These results suggest that the physiological adaptations that improve VO<sub>2max</sub> differ from those that allow to approach VO<sub>2VT1</sub> and VO<sub>2VT2</sub> to VO<sub>2max</sub> (i.e., improve %VO<sub>2max</sub>VT1 and %VO<sub>2max</sub>VT2). We theorized that when training is specifically designed to improve VO<sub>2max</sub>, the gains in VO<sub>2max</sub> could surpass those in VO<sub>2VT1</sub> or VO<sub>2VT2</sub>. In this case, VO<sub>2max</sub> may be diverging from VO<sub>2VT1</sub> or VO<sub>2VT2</sub>, lowering %VO<sub>2max</sub>VT1 and VT2. Conversely, when training is targeted to improve VO<sub>2VT1</sub> and VO<sub>2VT2</sub>, gains in these thresholds may exceed those in VO<sub>2max</sub>, increasing %VO<sub>2max</sub>VT1 and VT2.

We found that %VO<sub>2max</sub>VT1 and VT2 increased with higher levels of the aerobic fitness level. This suggests that, as the aerobic fitness level improves, there may be proportionally greater gains (or higher responsiveness) in VO<sub>2VT1</sub> and VO<sub>2VT2</sub> compared to VO<sub>2max</sub>. This leads us to propose that the variables most sensitive

to training (VO<sub>2VT1</sub> and VO<sub>2VT2</sub>) are better indicators of its effects and, therefore, may be the most suitable for assessing the impact of a training program. We suggest that the greater responsiveness of VO<sub>2VT1</sub> and VO<sub>2VT2</sub> compared to VO<sub>2max</sub> could be explained by the principle of training specificity, which suggests that adaptations mainly occur at the intensity level at which training is focused. Since endurance athletes typically follow a pyramidal distribution of training intensity (with a decreasing volume from moderate to heavy to severe zones) (Casado et al. 2022; Zapico et al. 2007), it is reasonable to assume that VO<sub>2VT1</sub> or VO<sub>2VT2</sub> would show greater improvements than VO<sub>2max</sub>, although this has been questioned by a recent study where the VO<sub>2VT1</sub> improved similarly after training in the heavy, severe, or extreme domain (Inglis et al. 2024). Another possible explanation is the differing influence of genetics on VO<sub>2max</sub> versus VO<sub>2VT1</sub> or VO<sub>2VT2</sub>. Evidence suggests a substantial genetic contribution to VO<sub>2max</sub> (Bouchard et al. 1999, 2011; Williams et al. 2017), whereas the genetic impact on VO<sub>2VT1</sub> and VO<sub>2VT2</sub> is less certain.

**TABLE 2** | Differences according to the aerobic fitness level on the different variables related to the heart rate.

|                            | Aerobic fitness level | N   | Mean | ± | SD                  | Aerobic fitness level effect                          |
|----------------------------|-----------------------|-----|------|---|---------------------|-------------------------------------------------------|
| Males                      |                       |     |      |   |                     |                                                       |
| HR <sub>VT1</sub> (bpm)    | Low                   | 315 | 122  | ± | 14.7                | $F(2,1432) = 98.7$ $p < 0.001$<br>$\eta^2 p = 0.121$  |
|                            | Medium                | 698 | 135  | ± | 13.9 <sup>a</sup>   |                                                       |
|                            | High                  | 424 | 144  | ± | 12.7 <sup>a,b</sup> |                                                       |
| HR <sub>VT2</sub> (bpm)    | Low                   | 312 | 157  | ± | 14.9                | $F(2,1430) = 63.7$ $p < 0.001$<br>$\eta^2 p = 0.082$  |
|                            | Medium                | 699 | 167  | ± | 12.2 <sup>a</sup>   |                                                       |
|                            | High                  | 424 | 176  | ± | 10.5 <sup>a,b</sup> |                                                       |
| HR <sub>max</sub> (bpm)    | Low                   | 311 | 177  | ± | 14                  | $F(2,1428) = 16.9$ $p < 0.001$<br>$\eta^2 p = 0.023$  |
|                            | Medium                | 699 | 183  | ± | 12.1                |                                                       |
|                            | High                  | 423 | 192  | ± | 10.7 <sup>a,b</sup> |                                                       |
| %HR <sub>max</sub> VT1 (%) | Low                   | 308 | 69.4 | ± | 6.5                 | $F(2,1423) = 72.42$ $p < 0.001$<br>$\eta^2 p = 0.092$ |
|                            | Medium                | 697 | 73.4 | ± | 6.3 <sup>a</sup>    |                                                       |
|                            | High                  | 423 | 75   | ± | 6.4 <sup>a,b</sup>  |                                                       |
| %HR <sub>max</sub> VT2 (%) | Low                   | 308 | 88.8 | ± | 4.8                 | $F(2,1424) = 46.4$ $p < 0.001$<br>$\eta^2 p = 0.061$  |
|                            | Medium                | 699 | 91.4 | ± | 4.4 <sup>a</sup>    |                                                       |
|                            | High                  | 422 | 91.6 | ± | 4.5 <sup>a</sup>    |                                                       |
| Females                    |                       |     |      |   |                     |                                                       |
| HR <sub>VT1</sub> (bpm)    | Low                   | 107 | 131  | ± | 15                  | $F(1,223) = 22.2$ $p < 0.001$<br>$\eta^2 p = 0.09$    |
|                            | High                  | 120 | 143  | ± | 15 <sup>a</sup>     |                                                       |
| HR <sub>VT2</sub> (bpm)    | Low                   | 105 | 165  | ± | 12                  | $F(1,221) = 12.1$ $p < 0.001$<br>$\eta^2 p = 0.052$   |
|                            | High                  | 120 | 172  | ± | 11 <sup>a</sup>     |                                                       |
| HR <sub>max</sub> (bpm)    | Low                   | 104 | 178  | ± | 13                  | $F(1,220) = 8.64$ $p < 0.001$<br>$\eta^2 p = 0.038$   |
|                            | High                  | 120 | 185  | ± | 11 <sup>a</sup>     |                                                       |
| %HR <sub>max</sub> VT1 (%) | Low                   | 101 | 74.4 | ± | 6.4                 | $F(1,215) = 16.79$ $p < 0.001$<br>$\eta^2 p = 0.072$  |
|                            | High                  | 118 | 77.9 | ± | 6 <sup>a</sup>      |                                                       |
| %HR <sub>max</sub> VT2 (%) | Low                   | 100 | 93.3 | ± | 4.2                 | $F(2,213) = 0.34$ $p = 0.558$<br>$\eta^2 p = 0.002$   |
|                            | High                  | 117 | 93.6 | ± | 3.3                 |                                                       |

Note: VT1 and VT2: ventilatory thresholds 1 and 2, respectively; HR<sub>VT1</sub>, HR<sub>VT2</sub>, and HR<sub>max</sub>: heart rate at VT1, VT2, and maximum, respectively; %HR<sub>max</sub>VT1 and %HR<sub>max</sub>VT2: percentage of the maximal heart rate at VT1 and VT2, respectively.

<sup>a</sup>Significant difference from a low aerobic fitness level.

<sup>b</sup>Significant difference from a medium aerobic fitness level.

These results showing a higher %VO<sub>2max</sub>VT1 and %VO<sub>2max</sub>VT2 as aerobic fitness level increases are consistent with a previous study on treadmill (Benítez-Muñoz et al. 2024). Our findings are consistent with a previous study reporting higher %VO<sub>2max</sub>VT1 and %VO<sub>2max</sub>VT2 values in well-trained compared to recreationally trained runners (Hetlelid et al. 2015). Additionally, our results align with another study showing that %VO<sub>2max</sub>VT1 and %VO<sub>2max</sub>VT2 varied across professional, U23, and junior cyclists (Alejo et al. 2022). They also concur with findings indicating that %VO<sub>2max</sub>VT1 and %VO<sub>2max</sub>VT2 increased as the training season progressed (Zapico et al. 2007). In contrast, a separate study found that the %VO<sub>2max</sub> at which the lactate threshold occurred was similar among recreational, national, and elite runners (Støa et al. 2020). Moreover, Iannetta et al. further specified that the %VO<sub>2max</sub> at which both the lactate threshold and maximal lactate steady state occurred did not vary among individuals with different aerobic fitness levels, based on the absence of correlation between VO<sub>2max</sub> and the

%VO<sub>2max</sub> at which these thresholds occurred (Iannetta et al. 2020). In our study, we also observed this lack of correlation between VO<sub>2max</sub> with %VO<sub>2max</sub>VT1 or %VO<sub>2max</sub>VT2. Iannetta et al. suggested that this absence of correlation might result from the limited VO<sub>2max</sub> range in their sample; however, this does not seem to be the case in our study, as we observed the same lack of correlation across a much broader VO<sub>2max</sub> range. Moreover, Iannetta et al. accurately noted that this lack of correlation could arise because the physiological determinants of VO<sub>2max</sub> differ from those of the thresholds. We further explored this by analyzing the association between VO<sub>2VT1</sub> and VO<sub>2VT2</sub> with %VO<sub>2max</sub>VT1 and %VO<sub>2max</sub>VT2, respectively, showing a positive association. These findings indicate that %VO<sub>2max</sub>VT1 and %VO<sub>2max</sub>VT2 are more closely linked to VO<sub>2VT1</sub> and VO<sub>2VT2</sub>, respectively, than to VO<sub>2max</sub>. Overall, based on our results, individuals with a higher specific aerobic fitness level at VT1 and VT2 exhibit a rightward shift relative to %VO<sub>2max</sub> in the location of these respective

**TABLE 3** | Differences according to the aerobic fitness level on the different variables related to power output.

|                           | Aerobic fitness level | N   | Mean  | ± | SD                  | Aerobic fitness level effect                            |
|---------------------------|-----------------------|-----|-------|---|---------------------|---------------------------------------------------------|
| Males                     |                       |     |       |   |                     |                                                         |
| W <sub>VT1</sub> (w)      | Low                   | 231 | 122.7 | ± | 32.7                | F (2,904) = 404.2 p < 0.001<br>η <sup>2</sup> p = 472   |
|                           | Medium                | 400 | 162.6 | ± | 34.8 <sup>a</sup>   |                                                         |
|                           | High                  | 277 | 216.2 | ± | 41.9 <sup>a,b</sup> |                                                         |
| W <sub>VT2</sub> (w)      | Low                   | 232 | 227   | ± | 49                  | F (2,904) = 366.5 p < 0.001<br>η <sup>2</sup> p = 0.448 |
|                           | Medium                | 400 | 274.9 | ± | 45.4 <sup>a</sup>   |                                                         |
|                           | High                  | 276 | 340.6 | ± | 44.4 <sup>a,b</sup> |                                                         |
| W <sub>max</sub> (w)      | Low                   | 312 | 289.1 | ± | 57.7                | F (2,1419) = 233 p < 0.001<br>η <sup>2</sup> p = 0.247  |
|                           | Medium                | 691 | 324.2 | ± | 56.6 <sup>a</sup>   |                                                         |
|                           | High                  | 421 | 388.2 | ± | 70.4 <sup>a,b</sup> |                                                         |
| %W <sub>max</sub> VT1 (%) | Low                   | 228 | 42.6  | ± | 9.4                 | F (2,895) = 72.86 p < 0.001<br>η <sup>2</sup> p = 0.14  |
|                           | Medium                | 397 | 48.9  | ± | 7.8 <sup>a</sup>    |                                                         |
|                           | High                  | 274 | 52.3  | ± | 7.2 <sup>a,b</sup>  |                                                         |
| %W <sub>max</sub> VT2 (%) | Low                   | 225 | 77.8  | ± | 11.2                | F (2,885) = 13.5 p < 0.001<br>η <sup>2</sup> p = 0.03   |
|                           | Medium                | 391 | 82.4  | ± | 6.8 <sup>a</sup>    |                                                         |
|                           | High                  | 273 | 82.8  | ± | 5.7 <sup>a</sup>    |                                                         |
| Females                   |                       |     |       |   |                     |                                                         |
| W <sub>VT1</sub> (w)      | Low                   | 66  | 80.3  | ± | 25.3                | F (1,125) = 66.84 p < 0.001<br>η <sup>2</sup> p = 0.348 |
|                           | High                  | 62  | 123   | ± | 34.7 <sup>a</sup>   |                                                         |
| W <sub>VT2</sub> (w)      | Low                   | 66  | 133.2 | ± | 38.3                | F (1,126) = 82.11 p < 0.001<br>η <sup>2</sup> p = 0.395 |
|                           | High                  | 63  | 206.9 | ± | 51.7 <sup>a</sup>   |                                                         |
| W <sub>max</sub> (w)      | Low                   | 101 | 188.1 | ± | 53.6                | F (1,211) = 19.65 p < 0.001<br>η <sup>2</sup> p = 0.085 |
|                           | High                  | 114 | 218.5 | ± | 55.6 <sup>a</sup>   |                                                         |
| %W <sub>max</sub> VT1 (%) | Low                   | 65  | 47    | ± | 10.2                | F (1,120) = 4.93 p = 0.028<br>η <sup>2</sup> p = 0.039  |
|                           | High                  | 58  | 51.5  | ± | 8.2 <sup>a</sup>    |                                                         |
| %W <sub>max</sub> VT2 (%) | Low                   | 66  | 78.6  | ± | 16.6                | F (1,121) = 7.12 p = 0.009<br>η <sup>2</sup> p = 0.056  |
|                           | High                  | 58  | 85.9  | ± | 6 <sup>a</sup>      |                                                         |

Note: VT1 and VT2: ventilatory thresholds 1 and 2, respectively;  $W_{VT1}$ ,  $W_{VT2}$ , and  $W_{max}$ : power output at VT1, VT2, and maximum, respectively; % $W_{max}VT1$  and % $W_{max}VT2$ : percentage of maximal power output at VT1 and VT2, respectively.

<sup>a</sup>Significant difference from a low aerobic fitness level.

<sup>b</sup>Significant difference from a medium aerobic fitness level.

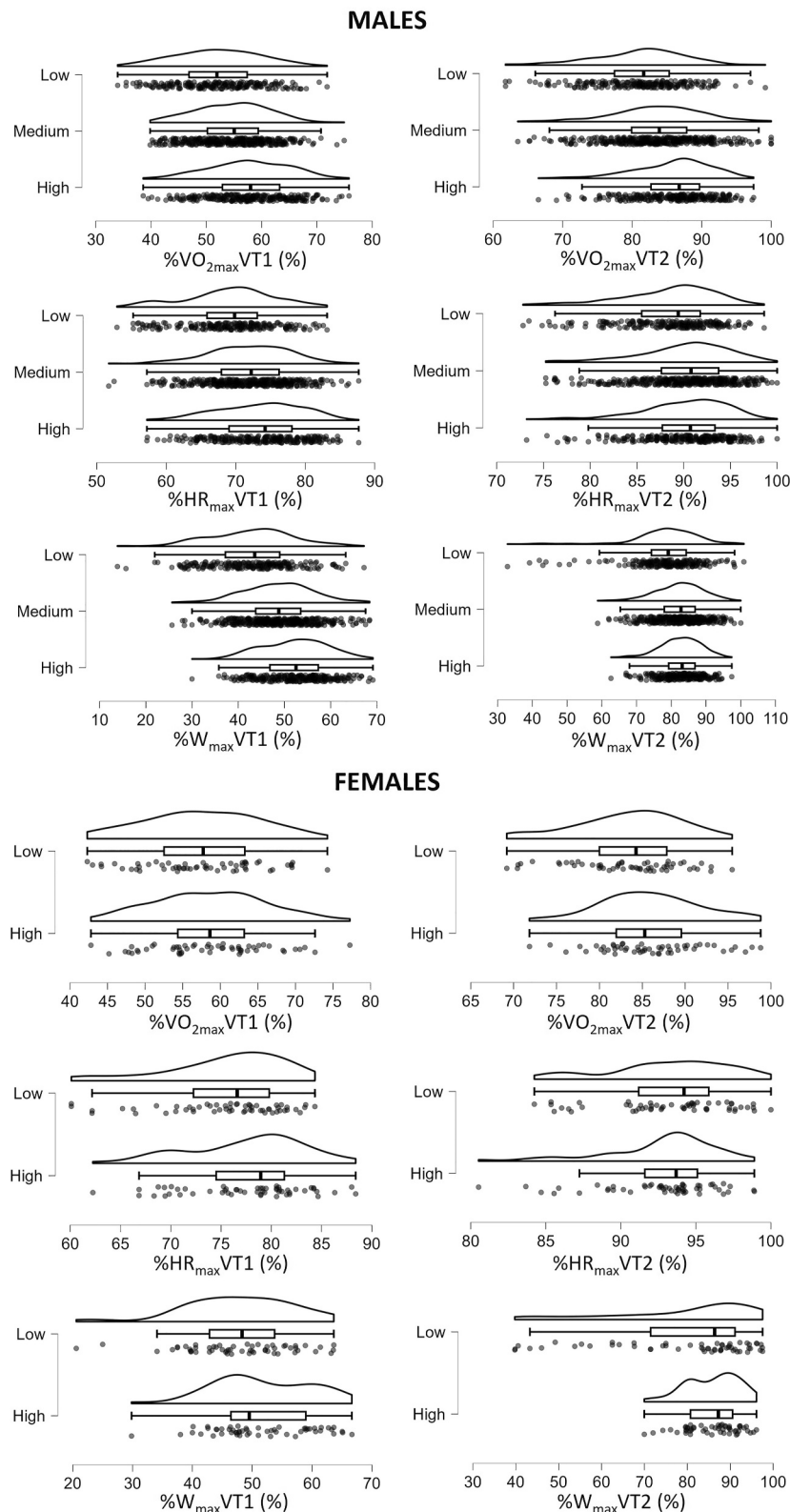
thresholds. However, we do not recommend evaluating the aerobic fitness level solely based on % $VO_{2max}VT1$  and % $VO_{2max}VT2$ , as a higher % $VO_{2max}VT1$  and % $VO_{2max}VT2$  could result from a lower  $VO_{2max}$  with similar  $VO_{2VT1}$  or  $VO_{2VT2}$  values, which does not necessarily indicate an improvement in the aerobic fitness level. Therefore, we recommend observing changes in % $VO_{2max}VT1$  and % $VO_{2max}VT2$  in parallel with  $VO_{2VT1}$ ,  $VO_{2VT2}$ , and  $VO_{2max}$ . The observed variability in the % $VO_{2max}VT1$  and % $VO_{2max}VT2$  even stratifying the sample by aerobic fitness levels and sex do not align with general guidelines that recommend exercise intensity based on fixed percentages of  $VO_{2max}$  (Garber et al. 2011; Haskell et al. 2007), as a specific percentage of  $VO_{2max}$  may lead to different physiological responses as % $VO_{2max}VT1$  and % $VO_{2max}VT2$  differ between aerobic fitness levels and sex.

Since exercise intensity is also often expressed as a fixed percentage of  $HR_{max}$  or  $W_{max}$ , we investigated whether the aerobic

fitness level affects % $HR_{max}VT1$  and  $VT2$  or % $W_{max}VT1$  and  $VT2$  in the same way as % $VO_{2max}VT1$  or  $VT2$ . Indeed, differences were found in % $HR_{max}VT1$  and  $VT2$  or % $W_{max}VT1$  and  $VT2$  between the aerobic fitness level as well, in agreement with previous studies (Benítez-Muñoz et al. 2024). Thus, establishing exercise intensity as a fixed percentage of maximum, regardless of whether  $VO_{2max}$ ,  $HR_{max}$ , or  $W_{max}$  is used, might actually elicit a different physiological response across individuals with varying aerobic fitness levels.

#### 4.1 | Methodological Considerations

As any study, the present one is not without considerations. One is that participants completed different incremental ramp exercise protocols tailored to their aerobic fitness levels. However, variations in the slope of load increment during the test do not appear to impact  $VO_{2VT1}$ ,  $VO_{2VT2}$ , or  $VO_{2max}$  (Iannetta



**FIGURE 2** | Ventilatory thresholds 1 and 2 position when expressed as  $\%VO_{2max}$ ,  $\%HR_{max}$ , or  $\%W_{max}$  according to the aerobic fitness level.  $\%VO_{2max}VT1$  and  $\%VO_{2max}VT2$ : percentage of maximal oxygen consumption at ventilatory thresholds 1 and 2, respectively;  $\%HR_{max}VT1$  and  $\%HR_{max}VT2$ : percentage of the maximal heart rate at ventilatory thresholds 1 and 2, respectively;  $\%W_{max}VT1$  and  $\%W_{max}VT2$  percentage of maximal power output at ventilatory thresholds 1 and 2, respectively.

et al. 2019; Leo et al. 2017; Scheuermann and Kowalchuk 1998), whereas excessively long protocols ( $> \sim 26$  min) may prevent reaching a true  $VO_{2max}$  due to fatigue (Midgley et al. 2008). This

is the main reason why participants in this study did not perform the same incremental test. Similarly, although protocols with steeper slopes yield higher workload values at

various physiological points (Jamnick et al. 2018), different slopes of load increment do not seem to affect  $HR_{VT1}$ ,  $HR_{VT2}$ , or  $HR_{max}$  (Scheuermann and Kowalchuk 1998). Regarding reference standard values, other studies with larger sample sizes classify participants by age and sex rather than by the aerobic fitness level (Loe et al. 2013; Vainshelboim et al. 2020). Although we acknowledge that our study has a smaller sample size, to our knowledge, this is the first study to date that reports reference values based on the aerobic fitness level. In this sense, there are considerably more males than females in our study, which implies that the results in males may better represent the population than those obtained in females. Aligned with that, the comparison between the values of an individual with those of the participants in the present study should only be made if the characteristics of the evaluated individual and the incremental test performed are very similar to the average characteristics of the present study. Another point to consider is that respiratory gas exchange was measured using two different analyzers. However, prior research has validated both analyzers used here (Perez-Suarez et al. 2018; Rietjens et al. 2001). Additionally, the shift from heavy to severe intensity may be indicated by other thresholds apart from VT2 (such as critical power or maximal lactate steady state). In this sense, although the power output at which critical power, maximal lactate steady state or VT2 occur may differ, the  $VO_2$  (the first variable in our study) and HR associated with these thresholds appears to be very similar (Iannetta et al. 2019; Keir et al. 2015). In this sense, it is feasible to compare CP, MLSS, and VT2 as long as the comparisons are made using  $VO_2$  or HR, rather than power output. Finally, as a cross-sectional study, we cannot confirm that training itself caused the differences observed between aerobic fitness levels. However, considering the outstanding sample size and the wide spectrum of  $VO_2$  included in the present article, conducting a training program with the current study's sample size would be highly impractical.

## 5 | Conclusion

$VO_{2VT1}$  and  $VO_{2VT2}$  present a positive and stronger association on  $\%VO_{2max}VT1$  and  $\%VO_{2max}VT2$ , respectively, compared to  $VO_{2max}$ , which has a very weak association. Therefore,  $VO_{2max}$  does not adequately distinguish certain differences in the aerobic fitness level, such as  $\%VO_{2max}VT1$  and VT2. Furthermore, our findings suggest that the  $\%VO_{2max}$ ,  $\%HR_{max}$ , and  $\%W_{max}$  at which thresholds occur are higher in individuals with a more advanced aerobic fitness level, classified combining  $VO_{2VT1}$ ,  $VO_{2VT2}$ , and  $VO_{2max}$ . Importantly, there is still a very high heterogeneity in the percentage of maximum at which VT1 and VT2 occur despite stratifying the participants by sex and aerobic fitness levels. These results indicate that the common practice of setting exercise intensity as a fixed percentage of maximum is not effective in aligning intensity even if sex and aerobic fitness levels is considered.

## Author Contributions

data curation: J.A.B.M., M.A.A., R.C., I.G.C., M.A.R.T., V.M.A.M., N.R.P., A.P.C., D.J.R.M., A.B.P. and P.B. formal analysis: J.A.B.M. funding

acquisition: A.B.P. and P.J.B. investigation: J.A.B.M., M.A.A., R.C., I.G.C., M.A.R.T., V.M.A.M., N.R.P., A.P.C., D.J.R.M., A.B.P. and P.J.B. methodology: J.A.B.M., M.A.A., R.C., I.G.C., M.A.R.T., V.M.A.M., N.R.P., A.P.C., D.J.R.M., A.B.P. and P.J.B. project administration: A.B.P. and P.J.B. resources: A.B.P. and P.J.B. supervision: A.B.P. and P.J.B. visualization: J.A.B.M. writing – original draft: J.A.B.M. writing – review and editing: M.A.A., R.C., I.G.C., M.A.R.T., V.M.A.M., N.R.P., A.P.C., D.J.R.M., A.B.P. and P.J.B.

## Acknowledgments

The authors of the present article would like to thank all the people who helped with the data collection during the whole history of the laboratory of exercise physiology of the LFE Research Group.

## Ethics Statement

The protocols and procedures were approved by the Ethical Principles for Medical Research Involving Human Subjects of the World Medical Association Declaration of Helsinki (1964) and further amendments. This study was approved by the Human Research Ethics Committee of the Universidad Politécnica de Madrid. The data and information obtained in the project were considered as confidential following the current Spanish legislation regulating personal data protection (Organic Law 3/2018). Therefore, the database was restricted to the researchers that participated in the study.

## Consent

All participants were carefully informed about the possible risk and benefits of the project and they provided written informed consent to participate in the study.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author, JABM, upon reasonable request.

## References

- Alejo, L. B., A. Montalvo-Pérez, P. L. Valenzuela, et al. 2022. "Comparative Analysis of Endurance, Strength and Body Composition Indicators in Professional, Under-23 and Junior Cyclists." *Frontiers in Physiology* 13. <https://doi.org/10.3389/fphys.2022.945552>.
- Benítez-Muñoz, J. A., P. J. Benito, I. Guisado-Cuadrado, R. Cupeiro, and A. B. Peinado. 2024. "Differences in the Ventilatory Thresholds in Treadmill According to Training Status in 971 Males and 301 Females: A Cross-Sectional Study." *European Journal of Applied Physiology*. <https://doi.org/10.1007/s00421-024-05622-z>.
- Benítez-Muñoz, J. A., M. Á. Rojo-Tirado, P. J. Benito Peinado, J. M. Murias, D. González-Lamuño, and R. Cupeiro. 2025. "Greater Relative First and Second Lactate Thresholds in Females Compared With Males: Consideration for Exercise Prescription." *International Journal of Sports Physiology and Performance* 20, no. 1: 30–36. <https://doi.org/10.1123/ijspp.2024-0079>.
- Bentley, D. J., J. Newell, and D. Bishop. 2007. "Incremental Exercise Test Design and Analysis: Implications for Performance Diagnostics in Endurance Athletes." *Sports Medicine* 37, no. 7: 575–586. <https://doi.org/10.2165/00007256-200737070-00002>.
- Bouchard, C., P. An, T. Rice, et al. 1999. "Familial Aggregation of  $VO_{2max}$  Response to Exercise Training: Results From the HERITAGE Family Study." *Journal of Applied Physiology* 87, no. 3: 1003–1008. <https://doi.org/10.1152/jappl.1999.87.3.1003>.

- Bouchard, C., M. A. Sarzynski, T. K. Rice, et al. 2011. "Genomic Predictors of the Maximal O<sub>2</sub> Uptake Response to Standardized Exercise Training Programs." *Journal of Applied Physiology* 110, no. 5: 1160–1170. <https://doi.org/10.1152/jappphysiol.00973.2010>.
- Byrd, B. R., J. Keith, S. M. Keeling, et al. 2019. "Personalized Moderate-Intensity Exercise Training Combined With High-Intensity Interval Training Enhances Training Responsiveness." *International Journal of Environmental Research and Public Health* 16, no. 12: 2088. <https://doi.org/10.3390/ijerph16122088>.
- Casado, A., F. González-Mohino, J. M. González-Ravé, and C. Foster. 2022. "Training Periodization, Methods, Intensity Distribution, and Volume in Highly Trained and Elite Distance Runners: A Systematic Review." *International Journal of Sports Physiology and Performance* 17, no. 6: 820–833. <https://doi.org/10.1123/ijssp.2021-0435>.
- Coates, A. M., M. J. Joyner, J. P. Little, A. M. Jones, and M. J. Gibala. 2023. "A Perspective on High-Intensity Interval Training for Performance and Health." *Sports Medicine* 53, no. S1: 85–96. <https://doi.org/10.1007/s40279-023-01938-6>.
- Cohen, J. 1988. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed. Lawrence Erlbaum Associates Inc. <https://doi.org/10.4324/9780203771587>.
- Garber, C. E., B. Blissmer, M. R. Deschenes, et al. 2011. "Quantity and Quality of Exercise for Developing and Maintaining Cardiorespiratory, Musculoskeletal, and Neuromotor Fitness in Apparently Healthy Adults: Guidance for Prescribing Exercise." *Medicine & Science in Sports & Exercise* 43, no. 7: 1334–1359. <https://doi.org/10.1249/MSS.0b013e318213febf>.
- Haskell, W. L., I. M. Lee, R. R. Pate, et al. 2007. "Physical Activity and Public Health: Updated Recommendation for Adults From the American College of Sports Medicine and the American Heart Association." *Circulation* 116, no. 9: 1081–1093. <https://doi.org/10.1161/CIRCULATIONAHA.107.185649>.
- Hetlelid, K. J., D. J. Plews, E. Herold, P. B. Laursen, and S. Seiler. 2015. "Rethinking the Role of Fat Oxidation: Substrate Utilisation During High-Intensity Interval Training in Well-Trained and Recreationally Trained Runners." *BMJ Open Sport and Exercise Medicine* 1, no. 1: 1–9. <https://doi.org/10.1136/bmjsem-2015-000047>.
- Hopkins, W. G., S. W. Marshall, A. M. Batterham, and J. Hanin. 2009. "Progressive Statistics for Studies in Sports Medicine and Exercise Science." *Medicine & Science in Sports & Exercise* 41, no. 1: 3–12. <https://doi.org/10.1249/MSS.0b013e31818cb278>.
- Iannetta, D., R. De Almeida Azevedo, D. A. Keir, and J. M. Murias. 2019. "Establishing the VO<sub>2</sub> Versus Constant-Work-Rate Relationship From Ramp-incremental Exercise: Simple Strategies for an Unsolved Problem." *Journal of Applied Physiology* 127, no. 6: 1519–1527. <https://doi.org/10.1152/jappphysiol.00508.2019>.
- Iannetta, D., E. C. Inglis, A. T. Mattu, et al. 2020. "A Critical Evaluation of Current Methods for Exercise Prescription in Women and Men." *Medicine & Science in Sports & Exercise* 52, no. 2: 466–473. <https://doi.org/10.1249/MSS.0000000000002147>.
- Iannetta, D., J. Zhang, J. M. Murias, and S. J. Aboodarda. 2022. "Neuromuscular and Perceptual Mechanisms of Fatigue Accompanying Task Failure in Response to Moderate-, Heavy-, Severe-, and Extreme-Intensity Cycling." *Journal of Applied Physiology* 133, no. 2: 323–334.
- Inglis, E. C., D. Iannetta, L. Rasica, et al. 2024. "Heavy-, Severe-, and Extreme-, But Not Moderate-Intensity Exercise Increase  $\dot{V}_{O2max}$  and Thresholds After 6 Wk of Training." *Medicine & Science in Sports & Exercise* 56, no. 7: 1307–1316. <https://doi.org/10.1249/MSS.0000000000003406>.
- Jamnick, N. A., J. Botella, D. B. Pyne, and D. J. Bishop. 2018. "Manipulating Graded Exercise Test Variables Affects the Validity of the Lactate Threshold and VO<sub>2peak</sub>." *PLoS One* 13, no. 7. <https://doi.org/10.1371/journal.pone.0199794>.
- Jamnick, N. A., R. W. Pettitt, C. Granata, D. B. Pyne, and D. J. Bishop. 2020. "An Examination and Critique of Current Methods to Determine Exercise Intensity." *Sports Medicine* 50, no. 10: 1729–1756. <https://doi.org/10.1007/s40279-020-01322-8>.
- Keir, D. A., F. Y. Fontana, T. C. Robertson, et al. 2015. "Exercise Intensity Thresholds: Identifying the Boundaries of Sustainable Performance." *Medicine & Science in Sports & Exercise* 47, no. 9: 1932–1940. <https://doi.org/10.1249/MSS.0000000000000613>.
- Keir, D. A., D. Iannetta, F. Mattioni Maturana, J. M. Kowalchuk, and J. M. Murias. 2022. "Identification of Non-Invasive Exercise Thresholds: Methods, Strategies, and an Online App." *Sports Medicine* 52, no. 2: 237–255. <https://doi.org/10.1007/s40279-021-01581-z>.
- Leo, J. A., S. Sabapathy, M. J. Simmonds, and T. J. Cross. 2017. "The Respiratory Compensation Point Is Not a Valid Surrogate for Critical Power." *Medicine & Science in Sports & Exercise* 49, no. 7: 1452–1460. <https://doi.org/10.1249/MSS.0000000000001226>.
- Loe, H., Ø. Rognmo, B. Saltin, and U. Wisløff. 2013. "Aerobic Capacity Reference Data in 3816 Healthy Men and Women 20–90 Years." *PLoS One* 8, no. 5: e64319. <https://doi.org/10.1371/journal.pone.0064319>.
- Mann, T., R. P. Lamberts, and M. I. Lambert. 2013. "Methods of Prescribing Relative Exercise Intensity: Physiological and Practical Considerations." *Sports Medicine* 43, no. 7: 613–625. <https://doi.org/10.1007/s40279-013-0045-x>.
- Meyler, S., L. Bottoms, and D. Muniz-Pumares. 2021. "Biological and Methodological Factors Affecting  $\dot{V}O_{2max}$  Response Variability to Endurance Training and the Influence of Exercise Intensity Prescription." *Experimental Physiology* 106: 1410–1424. <https://doi.org/10.1113/EP089565>.
- Meyler, S., L. Bottoms, D. Wellsted, and D. Muniz-Pumares. 2023. "Variability in Exercise Tolerance and Physiological Responses to Exercise Prescribed Relative to Physiological Thresholds and to Maximum Oxygen Uptake." *Experimental Physiology* 108, no. 4: 581–594. <https://doi.org/10.1113/EP090878>.
- Meyler, S. J. R., P. A. Swinton, L. Bottoms, et al. 2025. "Changes in Cardiorespiratory Fitness Following Exercise Training Prescribed Relative to Traditional Intensity Anchors and Physiological Thresholds: A Systematic Review With Meta-Analysis of Individual Participant Data." *Sports Medicine* 55, no. 2: 301–323. <https://doi.org/10.1007/s40279-024-02125-x>.
- Midgley, A. W., D. J. Bentley, H. Luttikholt, L. R. McNaughton, and G. P. Millet. 2008. "Challenging a Dogma of Exercise Physiology: Does an Incremental Exercise Test for Valid VO<sub>2max</sub> Determination Really Need to Last Between 8 and 12 Minutes?" *Sports Medicine* 38, no. 6: 441–447. <https://doi.org/10.2165/00007256-200838060-00001>.
- Perez-Suarez, I., M. Martin-Rincon, J. J. Gonzalez-Henriquez, et al. 2018. "Accuracy and Precision of the COSMED K5 Portable Analyser." *Frontiers in Physiology* 9, no. 1764. <https://doi.org/10.3389/fphys.2018.01764>.
- Poole, D. C., H. B. Rossiter, G. A. Brooks, and L. B. Gladden. 2021. "The Anaerobic Threshold: 50+ Years of Controversy." *Journal of Physiology* 599, no. 3: 737–767. <https://doi.org/10.1113/JP279963>.
- Preobrazenski, N., J. T. Bonafiglia, M. W. Nelms, et al. 2019. "Does Blood Lactate Predict the Chronic Adaptive Response to Training: A Comparison of Traditional and Talk Test Prescription Methods." *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition et Metabolisme* 44, no. 2: 179–186. <https://doi.org/10.1139/apnm-2018-0343>.
- Rabadán, M., V. Díaz, F. J. Calderón, P. J. Benito, A. B. Peinado, and N. Maffulli. 2011. "Physiological Determinants of Speciality of Elite Middle- and Long-Distance Runners." *Journal of Sports Sciences* 29, no. 9: 975–982. <https://doi.org/10.1080/02640414.2011.571271>.
- Rietjens, G. J., H. Kuipers, A. D. Kester, and H. A. Keizer. 2001. "Validation of a Computerized Metabolic Measurement System

- (Oxycon-Pro) During Low and High Intensity Exercise.” *International Journal of Sports Medicine* 22, no. 4: 291–294. <https://doi.org/10.1055/s-2001-14342>.
- Scheuermann, B. W., and J. M. Kowalchuk. 1998. “Attenuated Respiratory Compensation During Rapidly Incremented Ramp Exercise.” *Respiration Physiology* 114, no. 3: 227–238. [https://doi.org/10.1016/S0034-5687\(98\)00097-8](https://doi.org/10.1016/S0034-5687(98)00097-8).
- Sevilla-Lorente, R., A. Marmol-Perez, P. Gonzalez-Garcia, et al. 2024. “Sexual Dimorphism on the Acute Effect of Exercise in the Morning vs. Evening: A Randomized Crossover Study.” *Journal of Sport and Health Science* 14: 101021. <https://doi.org/10.1016/j.jshs.2024.101021>.
- Støa, E. M., J. Helgerud, B. R. Rønnestad, J. Hansen, S. Ellefsen, and Ø. Støren. 2020. “Factors Influencing Running Velocity at Lactate Threshold in Male and Female Runners at Different Levels of Performance.” *Frontiers in Physiology* 11. <https://doi.org/10.3389/fphys.2020.585267>.
- Vainshelboim, B., R. Arena, L. A. Kaminsky, and J. Myers. 2020. “Reference Standards for Ventilatory Threshold Measured With Cardiopulmonary Exercise Testing: The Fitness Registry and the Importance of Exercise: A National Database.” *Chest* 157, no. 6: 1531–1537. <https://doi.org/10.1016/j.chest.2019.11.022>.
- Weatherwax, R. M., N. K. Harris, A. E. Kilding, and L. C. Dalleck. 2019. “Incidence of V̇O<sub>2</sub>max Responders to Personalized Versus Standardized Exercise Prescription.” *Medicine & Science in Sports & Exercise* 51, no. 4: 681–691. <https://doi.org/10.1249/MSS.0000000000001842>.
- Wendler, T., and S. Grötrrup. 2021. *Data Mining With SPSS Modeler*. Springer International Publishing. <https://doi.org/10.1007/978-3-030-54338-9>.
- Williams, C. J., M. G. Williams, N. Eynon, et al. 2017. “Genes to Predict VO<sub>2</sub>max Trainability: A Systematic Review.” *BMC Genomics* 18, no. S8: 831. <https://doi.org/10.1186/s12864-017-4192-6>.
- Wolpern, A. E., D. J. Burgos, J. M. Janot, and L. C. Dalleck. 2015. “Is a Threshold-Based Model a Superior Method to the Relative Percent Concept for Establishing Individual Exercise Intensity? A Randomized Controlled Trial.” *BMC Sports Science, Medicine and Rehabilitation* 7, no. 1: 16. <https://doi.org/10.1186/s13102-015-0011-z>.
- Zapico, A. G., F. J. Calderón, P. J. Benito, et al. 2007. “Evolution of Physiological and Haematological Parameters With Training Load in Elite Male Road Cyclists: A Longitudinal Study.” *Journal of Sports Medicine and Physical Fitness* 47, no. 2: 191–196.