#### Research article

# Quantifying Running Economy in Amateur Runners: Evaluating VO<sub>2</sub> and Energy Cost with Model-based Normalization

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#### **Abstract**

Submaximal oxygen uptake (VO<sub>2sub</sub>) scaled by ratio is commonly used to evaluate running economy (RE) to reflect metabolic consumption at a given submaximal-intensity velocity. However, this method is questionable due to its neglect of substrate-related issues and the inherent mathematical discrepancies in ratio scaling. This study aimed to investigate the validity of ratio-scaled VO<sub>2sub</sub> as a measure of RE by comparing it with allometric-scaled energy cost (Ec, kcal/kgb/min). Sixty-nine recreationally active college students underwent VO<sub>2max</sub> tests and discontinuous submaximal running assessments at three %VO<sub>2max</sub> intensities. A 1000-meter test assessed running performance. One-way repeated-measures ANOVA compared changes in VO<sub>2sub</sub> or E<sub>c</sub> with increasing running intensities. Regression analysis explored methods for metabolic data standardization. Pearson correlation coefficient evaluated the effectiveness of standardization and the correlations between sports performance and RE scaled by different measures. Magnitude-based inferences were used to assess sex differences and probabilities of RE at each running intensity. Both VO2sub and Ec significantly increased with increasing intensities, suggesting that VO<sub>2sub</sub> is a valid quantification of RE. Allometric scaling is more suitable than ratio scaling for removing the influence of body weight on both Ec and VO2sub, with females showing better RE. Allometric-scaled Ec was sensitive in detecting correlations with performance, strongest at 65% VO<sub>2max</sub>. While VO<sub>2sub</sub> is a valid quantification of RE, allometric scaling, rather than ratio scaling, should be used to normalize the RE quantification before performing reliable interindividual comparisons. The 2/3 law can be considered as the exponent b value for body weight. Additionally, 65%VO<sub>2max</sub> intensity is recommended as the submaximal testing intensity in the RE test. Nonetheless, more studies with diverse samples are needed to confirm the validity.

**Key words:** Running economy, ratio scaling, oxygen consumption, energy cost, allometric scaling.

#### Introduction

Running economy (RE), defined as the metabolic consumption at a given submaximal-intensity velocity, is a more robust physiological indicator for distinguishing homogenous populations and predicting endurance performance than maximal oxygen uptake (VO<sub>2max</sub>) alone (Midgley et al., 2007; Shaw et al., 2014) .When combined with VO<sub>2max</sub> and lactate threshold or fractional utilization at the speed corresponding to lactate threshold (sLT), RE can explain up to 90% of the variance in distance events (Abad et al., 2016; Midgley et al., 2007). Therefore, ensuring the validity of RE measurements is crucial for evaluating human locomotion and performance.

Previous studies have examined inter-individual differences in specific anthropological, mechanical, and physiological aspects to identify factors contributing to an economical runner (Midgley et al., 2007; Segers et al., 2008; Shaw et al., 2014). A commonly used indirect measure of RE is submaximal oxygen uptake (VO<sub>2sub</sub>), expressed in milliliters per kilogram per minute (ml/kg/min) during steady-state exercise. This metric is based on the assumption that oxygen consumption represents adenosine triphosphate turnover during submaximal exercise, thereby serving as a proxy for overall energy expenditure (Shaw et al., 2014). However, the validity of VO<sub>2sub</sub> as a reliable quantification of RE has been questioned. Several studies have reported inconsistencies between changes in VO<sub>2sub</sub> and actual energy cost (E<sub>c</sub>) as running velocity increases (Fletcher et al., 2009; Shaw et al., 2014). One plausible explanation for this discrepancy is the metabolic shift toward greater carbohydrate utilization at higher exercise intensities. As intensity rises from approximately 55% to 75% of VO<sub>2max</sub>, there is increased recruitment of type II muscle fibers, thereby accompanied by a reduction in fat oxidation (from ~60% to ~35%) and a corresponding increase in carbohydrate oxidation (from ~35% to ~60%) (Jeukendrup and Wallis, 2005; Rapoport, 2010). This shift has important implications for interpreting oxygen consumption. Although carbohydrate oxidation requires less oxygen per gram (0.81 L) compared to fat oxidation (2.01 L), it also yields less energy (4.07 kcal vs. 9.75 kcal per gram) (Jeukendrup and Wallis, 2005). Consequently, during moderate to high-intensity exercise, total oxygen consumption may remain relatively stable despite increased energy output, potentially leading to misleading conclusions about RE when quantified by VO<sub>2sub</sub>. This phenomenon may partly explain the observed divergence between VO<sub>2sub</sub> and E<sub>c</sub> in previous studies (di Prampero et al., 2009; Fletcher et al., 2009; Jeukendrup and Wallis, 2005). Another factor that complicates the use of VO<sub>2sub</sub> as a quantification of RE is the variability in substrate utilization among individuals. For instance, if athlete A is more efficient at metabolizing fat than athlete B, athlete A may exhibit higher oxygen consumption due to the greater oxygen demand of fat oxidation (Hawley and Leckey, 2015). This could lead to the erroneous conclusion that athlete B has better RE than athlete A. Therefore, if oxygen consumption does not reflect the metabolic consumption well, ridiculous findings may occur, and the validity of VO<sub>2sub</sub> as a measure of RE could be compromised. Directly calculating energy consumption seems more accurate and reasonable, as it avoids issues related to substrate utilization and aligns with the definition

of RE. However, limited studies have employed  $E_{\rm c}$  as a primary measure of RE (Blagrove et al., 2017; di Prampero et al., 2009; Fletcher et al., 2009), and only a relatively small cohort of highly trained runners in these studies may weaken the power of relevant findings or not be suitable to be applied to other populations (e.g., non-athletes).

Moreover, accurate interindividual comparisons of RE require proper normalization of physiological variables, independent of potential confounding factors. Studies on the quantitative relationship between  $VO_{2max}$  and body weight (Armstrong and Welsman, 2019; Segers et al., 2008; Shaw et al., 2014) have observed a non-linear relationship and a significant negative correlation between VO<sub>2max</sub> / body weight, namely the normalized VO<sub>2max</sub>, and body weight. These findings challenge the validity of ratio scaling in removing the influence of body weight. Instead, allometric scaling  $(y = ax^b)$  seems more accurate to normalize physiological variables, where y represents VO<sub>2max</sub> and x represents body weight (Shingleton and Frankino, 2018). If ratio scaling is valid, the exponent b would equal 1, indicating a linear relationship. However, empirical studies on VO<sub>2max</sub> rejected the assumed linear relationship  $(b \neq 1)$ , with calculated b values clustering around either 2/3 or 3/4. These values correspond to established biological scaling laws (i.e., the 2/3 or 3/4 power scaling laws), leading scholars to suggest that an exponent b for body weight of 2/3 or 3/4 is acceptable when scaling VO<sub>2max</sub> (Chamari et al., 2005; Helgerud, 1994; Lolli et al., 2017). If a similar curvilinear relationship exists in scaling RE, findings related to RE expressed by ratio scaling should be viewed skeptically. For instance, results based on ratio scaling might lead to underestimation of an individual's aerobic capacity while overestimating its RE (Chamari et al., 2005; Davies et al., 1997; Lee et al., 2024; Lee and Zhang, 2021). Moreover, inconsistent results in RE differences between male and female athletes have been reported (Ariëns et al., 1997; Armstrong et al., 1999; Davies et al., 1997; Helgerud, 1994; Janz et al., 1998), which can be partly explained by the statistical adjustment method that is incapable of removing the influence of body weight when conducting inter-individual comparison. Despite the importance of this issue, relatively few studies(Armstrong et al., 1999; Fletcher et al., 2009; Lee et al., 2024; Rogers et al., 1995; Shaw et al., 2014; Welsman and Armstrong, 2000) have explored the relationship between body weight and RE measures. Moreover, it is inappropriate to apply the non-linear scaling relationship observed in VO<sub>2max</sub> directly to RE (Chamari et al., 2005), as the exponent b may vary depending on the metabolic state—shifting from resting to maximal conditions(Lee et al., 2024). Furthermore, these studies have focused on elite athletes, limiting the generalizability of their findings. The exploration in moderatelytrained runners with diverse athletic abilities may reflect more general characteristics of RE, specifically the universality of allometric-scaled E<sub>c</sub>.

Given that the relationship between body weight and measures of RE, as well as the application of these measures in sports performance, has yet to be examined in a larger cohort of recreationally active runners, the appropriate scaling of  $E_{\rm c}$  relative to body weight in this population remains to be elucidated. Therefore, this study

aimed to assess the validity of  $VO_{2sub}$  expressed by ratio scaling as a measure of RE in comparison with  $E_c$ . We hypothesized that allometric-scaled  $E_c$  would be a more appropriate RE measurement than the traditional ratio-scaled  $VO_{2sub}$ .

#### **Methods**

#### **Experimental design**

This study comprised different measurements assigned across four non-consecutive testing days spaced 1-7 days apart. On Day 1, anthropometric data (stature, body weight, gender, and age) and  $VO_{2max}$  were assessed. On Day 2, treadmill velocities eliciting 55%, 65%, and 75% $VO_{2max}$  for the RE test were verified. On Day 3, the formal RE assessment was conducted at these set speeds. On Day 4, a 1000-meter running field test was performed.

All tests were conducted in the afternoon, with the laboratory temperature maintained at 22-25 °C. Participants were required to avoid high-intensity exercises 24 hours before the tests to prevent fatigue. In addition, participants were instructed to fast for 2 hours before the tests and to wear the same sports shoes for all tests. Consumption of caffeine and tea beverages was prohibited before the tests.

#### **Participants**

Ethical approval of this study was obtained from the Human Research Ethics Committee of South China Normal University (SCNU-SPT-2022-040). The research was conducted in accordance with the principles of the Declaration of Helsinki and the local statutory requirements. Participants were recruited through campus advertisements according to the following criteria: (1) aged 18-30 years; (2) regular running habits, engaging in at least moderate-intensity running exercise three times a week for at least one hour per session; (3) free from any sports-related injuries or respiratory problems; (4) non-smoker and not habitual alcohol drinkers. Exclusion criteria included: experiencing any sports injury within the past 3 months, being a studentathlete, having physiological or psychological defects or diseases, regularly participating in sports/exercise activities other than running, or lacking exercise habits. Ultimately, sixty-nine recreationally active college students (34 males, age,  $23.9 \pm 4.7$  years, stature,  $174.4 \pm 5.4$  cm, body weight,  $66.5 \pm 6.6$  kg, BMI,  $21.4 \pm 3.1$  kg/m<sup>2</sup>; 35 females, age,  $22.0 \pm 2.1$  years, stature,  $159.8 \pm 5.7$  cm, body weight,  $53.3 \pm 6.9$  kg, BMI,  $20.7 \pm 2.0$  kg/m<sup>2</sup>) who met the criteria volunteered for the experiment. All participants were informed about the study procedures and expectations and provided written informed consent before participation.

#### Measurements

*VO<sub>2</sub>max test:* Based on our pre-test, a continuous incremental treadmill test adapted from (Werneck et al., 2019) was applied to measure VO<sub>2max</sub>. Participants performed a 10-minute warmup exercise at a velocity of 6 km/h and 0% slope before the formal test. After a 10-minute break, the formal test began with the velocity set at 8km/h and a slope of 4%. The velocity increased by 0.6 km/h every minute

until the destination of the test. When the respiratory exchange ratio (RER) approached 1, blood lactate was measured from the fingertip using EKF Lactate Scout+ (Barleben, Germany) in the last 30 seconds of each stage. A portable COSMED K5 metabolic analyzer (Rome, Italy) was used to collect relevant gas data. The criteria for  $VO_{2max}$  were based on the new proposals by (Edvardsen et al., 2014): 1) for females aged 20-49, blood lactate  $\geq 7.0$  mmol/L and the RER  $\geq 1.1$ ; 2) for males aged 20-49, blood lactate  $\geq 9.0$  mmol/L and RER  $\geq 1.1$ . For safety reasons, the test was also terminated if the participants reached volitional exhaustion and could not continue. Average oxygen uptake for the last 30 seconds was considered  $VO_{2max}$ .

Determining velocities for RE test: Given that VO<sub>2max</sub> is a widely recognized and standardized measure of aerobic capacity, using VO<sub>2max</sub> as a reference point allows for comparability across studies and populations (Mann et al., 2013). Furthermore, VO<sub>2max</sub> is generally more reliable than threshold measurements, such as lactate threshold, which can be complex, time-consuming, and challenging to implement consistently (Mann et al., 2013). Consequently, moderate-intensity exercise set at a percentage of VO<sub>2max</sub> is less likely to elicit individual variation in blood lactate accumulation (Mann et al., 2013). Therefore, many researchers favor using %VO<sub>2max</sub>, and the RE test set relative to VO<sub>2max</sub> in this study is reasonable. Given that submaximal intensity exercise typically corresponds to a range of 50% to 85% of VO<sub>2max</sub> (Jeukendrup and Wallis, 2005; Williams et al., 1991), we applied 55%, 65%, and 75% of VO<sub>2max</sub> to represent the submaximal intensities in RE testing. The velocities corresponding to 55%, 65%, and 75%VO<sub>2max</sub> were referred to the method of (Morgan and Daniels, 1994). Based on the data of velocities and corresponding oxygen consumption (60%-90%VO<sub>2max</sub>) collected from the  $VO_{2max}$  test, a linear model was established. Once the linear model was created, velocities corresponding to 55%, 65%, and 75%VO<sub>2max</sub> were initially calculated, followed by adjustment and determination in the laboratory. Participants were required to run at each calculated velocity for 6 minutes. The average oxygen consumption for the last 2 minutes was used to determine if the intensity triggered by the calculated velocities falls within 55%  $\pm$ 5%,  $65\% \pm 5\%$ , and  $75\% \pm 5\% VO_{2max}$ . If the intensity was outside the expected range, the calculated velocity was adjusted by 1km/h, and another 6-minute run was required to verify. The total trials should be no more than 5 times, and each 6-minute run was followed by a 10-minute break. The treadmill slope was set at 1% to simulate outdoor running conditions (Hung et al., 2019).

*RE test:* Comparisons based on absolute velocity neglect the differences in relative intensity (Fletcher et al., 2009) and therefore, %VO<sub>2max</sub> was applied to control the intensity in this study. Participants performed a 10-minute warm-up at 50%VO<sub>2max</sub> on a treadmill with a 0% slope. After a 5-minute resting period, the formal RE test began. Participants should run at the velocities corresponding to 55%, 65%, and 75% of VO<sub>2max</sub> on a treadmill set at a 1% slope for 6 minutes. The average oxygen uptake and carbon dioxide production during the last 2 minutes of each run were calculated and used to calculate the energy cost based on the updated conversion formulae proposed by

(Jeukendrup and Wallis, 2005). Blood lactate was collected immediately after each 6-minute run. A 5-minute standing rest was given before the next run. Steady-state criteria were based on suggestions by (Fletcher et al., 2009; McMiken and Daniels, 1976): 1) RER  $\leq$  0.95; 2) an increase of < 100 ml O<sub>2</sub> over the final 2 minutes of each stage. If the increase exceeded 100 ml, this run was extended by another 30 seconds.

Field test: A break of at least 1 day was given after the RE test, and the 1000-meter test was conducted on a standard athletic field within 7 days after the completion of the RE test. Before the test, participants performed a 5-minute 'quick warm-up cardio workout' followed by another 5-minute freestyle warm-up exercise. This 'quick warm-up cardio workout' included 10 different exercises (boxer shuffle, overhead reach + stretch, high knee march, torso twists, toe touch kicks, full torso circles, lateral step toe touches, squats, jumping jacks, high knees) with 30 seconds apiece. To simulate a competitive environment during the test, participants who had completed the laboratory tests competed in the field test together rather than individually. Strong verbal encouragement was provided by the same assistant throughout the test. Time was recorded by the same qualified referee using a stopwatch. The average outdoor temperature and relative humidity were  $27.3 \pm 3.0$  $^{\circ}$ C and  $78.2 \pm 2.9$  %, respectively.

#### Explanation for tests employed in this study

All the laboratory tests worked for the RE test. Although the slope used in VO<sub>2max</sub> testing (4%) differed from that employed in the RE test (1%), thereby complicating the determination of appropriate speed for the RE test, this protocol was adopted to ensure participant safety and to obtain reliable VO<sub>2max</sub> measurements, which is critical to determine relative exercise intensities. In the RE test, the intensity was controlled to ensure consistency among individuals. Therefore, %VO<sub>2max</sub> was applied to control the intensity, and the treadmill velocity varied for each participant. A continuous incremental treadmill test of VO<sub>2max</sub> was applied for each participant (Day 1), in which the treadmill velocity of each stage and the corresponding VO<sub>2sub</sub> were also collected. Using the method described by (Morgan and Daniels, 1994), a simple linear model was established, with the velocity as the predictor and the corresponding VO<sub>2sub</sub> as the outcome variable. This allowed for the preliminary calculation of treadmill velocities corresponding to %VO<sub>2max</sub>. However, due to limited data and different settings between the VO<sub>2max</sub> test and the RE test, these calculated velocities were only for reference and facilitated the selection of required velocities for each participant to reach the target intensities. They were not directly applied in the RE test. Therefore, a pre-test for adjusting and verifying these preliminary velocities was necessary (Day 2). Once adjusted and confirmed, these velocities were used in the formal RE test (Day 3).

#### **Energy consumption**

Energy consumption during the RE test was calculated using energy conversion formulae recommended by (Jeukendrup and Wallis, 2005). These formulae are based on indirect calorimetry, which assumes that the RER

adequately reflects the respiratory quotient (RQ) (Jeukendrup and Wallis, 2005). In plain language, oxygen consumption and carbon dioxide production are assumed to result solely from oxidative processes, and the gas composition measured in exhaled breath reflects gas exchanged from fuel metabolism at the tissue level. The indirect calorimetry technique for substrate oxidation determination, as described by (Jeukendrup and Wallis, 2005), has been proven reliable and valid for low- to moderate-intensity exercise using stable isotope techniques. Consequently, these energy conversion formulae can accurately calculate energy consumption from different substrates and provide a reliable measure of metabolic consumption during the RE test.

Carbohydrate oxidation  $(g/min) = 4.210VCO_2-2.962VO_2 \mathbf{1}$ 

Fat oxidation 
$$(g/min) = 1.695 \text{ VO}_2 - 1.701 \text{ VCO}_2$$

Energy cost (kcal/min) = 
$$0.550 \text{ VCO}_2 + 4.471 \text{ VO}_2$$
 3

#### Statistical analysis

Normality distribution was assessed by the Shapiro-Wilk test. Regression analysis based on the following equations was applied to explore the quantitative relationship between  $VO_{2sub}$  or  $E_c$  (averaged for the three intensities,  $55\%VO_{2max}$ ,  $65\%VO_{2max}$ , and  $75\%VO_{2max}$ ) and body weight.

*Linear function:* 
$$y = kx + d$$
 4

Allometric scaling: 
$$y = a x^b$$
 5

A natural logarithm transformation ( $\ln y = \ln a + b \ln x$ ) was performed for equation 5 before conducting linear regression to calculate the b value. Specifically, the natural logarithm can be taken on both sides of equation 5, which gives:

$$ln y = ln (a x^b)$$

According to the sum and difference formulas for logarithmic functions ( $\log_c m + \log_c n = \log_b (mn)$ ;  $\log_b m - \log_b n = \log_b (m/n)$ ), equation 6 can be changed to

$$ln y = ln a + b ln x$$

Let  $Y = \ln y$ ,  $X = \ln x$ ,  $C = \ln a$ , the equation 7 can be written as

$$Y = b X + C$$

Therefore, a linear regression was applied to calculate the b values. The probability of future similar studies observing the group differences and the probability of the exponent b being lower than 1 or the constant d being greater than 0 were interpreted according to the following scale (Hopkins and Batterham, 2016): <0.5%, most unlikely; 0.5-5%, very unlikely; 5-25%, unlikely; 25-75%, possible; 75-95%, likely; 95%-99.5%, very likely; >99.5%, most likely. Pearson Correlation Coefficient was applied to

verify the influence of body weight on measures of RE and their correlations with running performance. If the model could succeed in accounting for body weight, the correlation between body weight and the assessed variable scaled appropriately to body weight should not exist (Lolli et al., 2017). The correlation coefficient r was interpreted according to the following ratings (Landis and Koch, 1977): no correlation or trivial correlation,  $|\mathbf{r}| < 0.2$ ; fair,  $0.2 \le |\mathbf{r}| < 0.4$ ; moderate,  $0.4 \le |\mathbf{r}| < 0.6$ ; substantial,  $0.6 \le |\mathbf{r}| < 0.8$ ; almost perfect,  $|\mathbf{r}| \ge 0.8$ .

A one-way repeated-measures ANOVA was used to evaluate differences in VO<sub>2sub</sub> and E<sub>c</sub> as measures of RE across three velocities. Post hoc analysis with Bonferroni adjustment was applied to identify where any significant differences occurred. Magnitude-based inferences proposed by (Hopkins et al., 2009) were utilized to reveal RE differences between males and females, as they provide a more accurate and informative ternary result with probability and magnitude (e.g., possibly substantially positive, possibly trivial, possibly substantially negative), avoiding the 'all or nothing' thinking that p-values encourage. Cohen's D effect sizes (ES) were calculated to reflect the extent of the difference, with the inferences associated with the effects defined as trivial (<0.20), small (0.20-0.59), moderate (0.60-1.19), large (1.2-1.9), very large (2.0-3.9) and extremely large ( $\geq 4.0$ ). All the data were processed in SPSS (version 20.0., IBM, Chicago, USA). The significance level was set at 0.05.

#### Results

#### Metabolic characteristics under different intensities

Relevant metabolic and physiological information is provided in Table 1. The average  $VO_{2max}$  for males and females was  $3.78\pm0.43$  and  $2.35\pm0.44$  L/min, respectively. The average  $VO_{2sub}$  at  $55\%VO_{2max}$ ,  $65\%VO_{2max}$ , and  $75\%VO_{2max}$  were  $2.15\pm0.26$ ,  $2.48\pm0.27$ , and  $2.77\pm0.33$  L/min, respectively, for males, and  $1.35\pm0.25$ ,  $1.58\pm0.29$ , and  $1.85\pm0.32$  L/min, respectively, for females. The corresponding  $E_c$  for males were  $10.72\pm1.27$ ,  $12.39\pm1.35$ , and  $13.83\pm1.62$  kcal/min, respectively, while those for females were  $6.71\pm1.21$ ,  $7.87\pm1.42$ , and  $9.23\pm1.59$  kcal/min, respectively.

Moreover, based on equations 1 and 2, the fat utilization rate (g/min) and the corresponding percentage of energy derived from fat (fat%) were calculated. Specifically, concerning the fat utilization rate in males, it experienced an increase from 0.18 g/min (55%VO<sub>2max</sub>) to 0.23 g/min (65%VO<sub>2max</sub>) before decreasing back to 0.18 g/min (75%VO<sub>2max</sub>). In contrast, this rate consistently decreased in females, experiencing a slight decrease from 0.26 g/min (55%VO<sub>2max</sub>) to 0.24 g/min (65%VO<sub>2max</sub>) and ultimately reaching 0.19 g/min at 75%VO<sub>2max</sub>. Although fat% for males was relatively stable when the intensity increased from  $55\%VO_{2max}$  to  $65\%VO_{2max}$ , it still displayed a decreasing trend in both males and females (male: 16%, 18%, and 13% at 55%VO<sub>2max</sub>, 65%VO<sub>2max</sub>, and 75%VO<sub>2max</sub>, respectively; female: 37%, 31%, and 20% at 55%VO<sub>2max</sub>, 65%VO<sub>2max</sub>, and 75%VO<sub>2max</sub>, respectively). Together, these suggested the metabolic shift towards carbohydrates as intensity increased.

table 1. Metabone and physiological characteristics: Data are means = 5D.							
Intensity		VO <sub>2sub</sub> (L/min)	VO <sub>2max</sub> (L/min)	Ec (kcal/min)	Blood lactate (mmol/L)	RER	
55%VO <sub>2max</sub>	Male	2.15±0.26	-	$10.72\pm1.27$	2.56±1.12 (range: 1.3-5.3)	$0.95\pm0.08$	
	Female	$1.35\pm0.25$	-	$6.71\pm1.21$	2.09±0.93 (range: 0.7-4.5)	$0.89\pm0.06$	
65%VO <sub>2max</sub>	Male	$2.48\pm0.27$	-	12.39±1.35	2.82±1.11 (range: 1.1-5.8)	$0.94\pm0.06$	
	Female	$1.58\pm0.29$	-	$7.87\pm1.42$	2.24±1.18 (range: 0.7-4.9)	$0.91\pm0.06$	
75%VO <sub>2max</sub>	Male	$2.77\pm0.33$	-	$13.83\pm1.62$	3.24±1.01 (range: 0.8-5.1)	$0.96\pm0.07$	
	Female	$1.85\pm0.32$	-	$9.23\pm1.59$	2.82±0.98 (range: 1.0-4.9)	$0.94\pm0.07$	
VO <sub>2max</sub>	Male	-	$3.78\pm0.43$		13.8±4.1	1.11±0.06	
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Table 1. Metabolic and physiological characteristics. Data are means  $\pm$  SD.

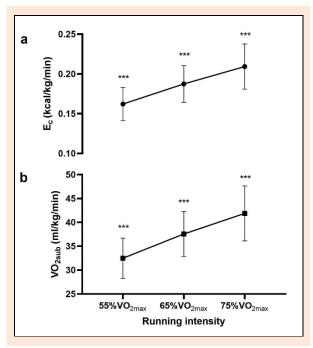


Figure 1. Physiological measures for males at three speeds relative to  $\text{%VO}_{2\text{max}}$ . \*\*\* Significant difference from all other intensities (ANOVA with Bonferroni post hoc adjustment; p < 0.001.

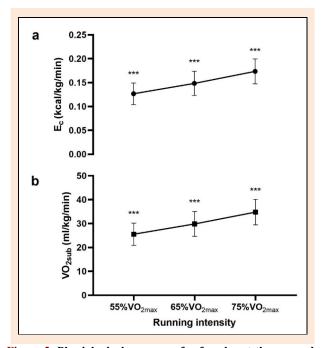


Figure 2. Physiological measures for females at three speeds relative to  $\text{%VO}_{2\text{max}}$ . \*\*\* Significant difference from all other intensities (ANOVA with Bonferroni post hoc adjustment; p < 0.001).

## Increased velocity and characteristics of different RE measures

The one-way repeated-measures ANOVA revealed increases in  $E_c$  with increments in running intensity, regardless of sex (p < 0.001; Figure 1a and Figure 2a). Increases in  $VO_{2sub}$  were also observed with increasing running intensity, regardless of sex (ANOVA, p < 0.001; Figure 1b and Figure 2b).

# Regression of RE with different measures under different intensities

In this study, "boverall" is defined as the exponent b value calculated from the relationship between body weight and VO<sub>2sub</sub> or E<sub>c</sub> averaged across three intensities. The results of regression analysis with allometric scaling showed significant fits between absolute E<sub>c</sub> and body weight (males: averaged across three intensities,  $b_{overall} = 0.48$ , 95%CI = 0.11-0.85; 55%VO<sub>2max</sub>, b = 0.53, 95%CI = 0.14-0.92;  $65\%VO_{2max}$ , b = 0.48, 95%CI = 0.10-0.87;  $75\%VO_{2max}$ , b = 0.44,95%CI = 0.04-0.85; females: averaged across three intensities,  $b_{overall} = 0.71$ , 95%CI = 0.28-1.14; 55%VO<sub>2max</sub>,  $b = 0.63, 95\%CI = 0.14-1.11; 65\%VO_{2max}, b = 0.67, 95\%CI$ = 0.20-1.14; 75%VO<sub>2max</sub>, b = 0.81, 95%CI = 0.38-1.23) and between VO<sub>2sub</sub> and body weight (males: averaged across three intensities,  $b_{overall} = 0.47$ , 95%CI = 0.10-0.85;  $55\%VO_{2max}$ , b = 0.52, 95%CI = 0.13-0.91;  $65\%VO_{2max}$ , b = 0.48, 95%CI = 0.09-0.89; 75%VO<sub>2max</sub>, b = 0.43, 95%CI = 0.03-0.84; females: averaged across three intensities, b<sub>o</sub>- $_{\text{verall}} = 0.71, 95\%\text{CI} = 0.27\text{-}1.15; 55\%\text{VO}_{2\text{max}}, b = 0.63,$ 95%CI = 0.14-1.13; 65%VO<sub>2max</sub>, b = 0.68, 95%CI = 0.20-1.15;  $75\%VO_{2max}$ , b = 0.79, 95%CI = 0.36-1.22). For future similar studies, the probability of exponent boverall (derived from E<sub>c</sub> and body weight) being less than 1 was 99.6% (most likely) in males and 90.7% (likely) in females. Furthermore, the probabilities of exponent b (derived from E<sub>c</sub> and body weight) being less than 1 at specific exercise intensities was as follow: in males, 98.9% (very likely) at 55%VO<sub>2max</sub>, 99.5% (most unlikely) at 65%VO<sub>2max</sub>, and 99.6% (most likely) at 75%VO<sub>2max</sub>; in females, 93.7% (likely) at 55%VO<sub>2max</sub>, 91.9% (likely) at 65%VO<sub>2max</sub>, and 82% (likely) at 75%VO<sub>2max</sub>. Likewise, the probabilities of the exponent boverall (calculated from VO2sub and body weight) being less than 1 in future similar studies were 99.6% for males (most likely) and 91.0% for females (likely). Furthermore, the probabilities of exponent b (derived from VO<sub>2sub</sub> and body weight) being less than at specific exercise intensities was as follow: in males, 99.1% (very likely) at 55%VO<sub>2max</sub>, 99.5% (very likely) at 65%VO<sub>2max</sub>, and 99.6% (most likely) at 75%VO<sub>2max</sub>; in females, and 93.0% (likely) at 55%VO<sub>2max</sub>, 91.9% (likely) at 65%VO<sub>2max</sub>, and 82.3% (likely) at 75%VO<sub>2max</sub>. Similar but

marginally higher  $R^2$  values were observed for allometric scaling compared to the linear function, regardless of whether the dependent variable was  $E_c$  or  $VO_{2sub}$ . Furthermore,  $R^2$  was consistently higher when allometric scaling was applied with  $E_c$  as the dependent variable and body weight as the independent variable, compared to when  $VO_{2sub}$  was the dependent variable and body weight was the independent variable (Table 2).

#### Removal effect of body weight

The appropriateness of allometric scaling was confirmed by the absence of any relationship when body weight was replotted against power-scaled  $E_c$  (kcal/kgb/min) and  $VO_{2sub}$  (ml/kgb/min). In contrast, the significant negative correlations between ratio-scaled  $E_c$  (kcal/kg/min) or ratio-scaled  $VO_{2sub}$  (ml/kg/min) and body weight in males suggested the failure of ratio scaling in removing the influence of body weight. Although the correlations were not significant in females when body weight was replotted against ratio-scaled  $E_c$  (kcal/kg/min) and  $VO_{2sub}$  (ml/kg/min), there was still a trend towards trivial or even fair negative correlations (Table 3).

#### Correlations between RE and running performance

The 1000 meters running performance for males was  $224.71 \pm 17.10$  s, while the performance for females was  $299.92 \pm 33.53$  s.

Table 4 displays the correlations between RE expressed by different measures and running performance. Regarding correlations between allometric-scaled  $E_c$  and performance in males, significantly positive correlations were observed at  $55\%VO_{2max}$  (r=0.43 [moderate]),  $65\%VO_{2max}$  (r=0.51 [moderate]),  $75\%VO_{2max}$  (r=0.50 [moderate]), and overall intensity (averaged across three intensities, r=0.51 [moderate]). In females, however, significantly positive correlations were observed at  $55\%VO_{2max}$  (r=0.35 [fair]),  $65\%VO_{2max}$  (r=0.46 [moderate]), and overall intensity (r=0.38 [fair]). Regarding correlations between ratio-scaled  $E_c$  and performance in males, significantly positive correlations were observed at  $55\%VO_{2max}$  (r=0.35 [fair]),  $65\%VO_{2max}$  (r=0.41 [moderate]),  $75\%VO_{2max}$  (r=0.41 [moderate]), and overall intensity.

sity (r = 0.41 [moderate]). In females, however, significantly positive correlations were observed at 55%VO<sub>2max</sub>  $(r = 0.34 \text{ [fair]}), 65\% VO_{2max} (r = 0.45 \text{ [moderate]}), and$ overall intensity (r = 0.37 [fair]). Regarding correlations between allometric-scaled VO<sub>2sub</sub> and performance in males, significantly positive correlations were observed at  $55\%VO_{2max}$  (r = 0.42 [moderate]),  $65\%VO_{2max}$  (r = 0.51 [moderate]),  $75\%VO_{2max}$  (r = 0.50 [moderate]), and overall intensity (r = 0.51 [moderate]). In females, however, significantly positive correlations were observed at  $55\%VO_{2max}$  (r = 0.35 [fair]),  $65\%VO_{2max}$  (r = 0.45 [moderate]), and overall intensity (r = 0.37 [fair]). Regarding correlations between ratio-scaled VO<sub>2sub</sub> and performance in males, significantly positive correlations were observed at  $55\%VO_{2max}$  (r = 0.35 [fair]),  $65\%VO_{2max}$  (r = 0.41 [moderate]),  $75\%VO_{2max}$  (r = 0.40 [moderate]), and overall intensity (r = 0.41 [moderate]). In females, however, significantly positive correlations were observed at  $65\%VO_{2\text{max}}$ (r = 0.44 [moderate]), and overall intensity (r = 0.36 [fair]). A fair but nonsignificant correlation (r = 0.33) between ratio-scaled VO<sub>2sub</sub> (at 55%VO<sub>2max</sub>) and performance was observed in females. Likewise, at 75%VO<sub>2max</sub>, none of the expressions detected significant correlations in females, although their correlations were fair. Overall, RE measured by E<sub>c</sub> consistently showed a marginally higher correlation with running performance and was more sensitive in detecting correlations with performance compared to VO<sub>2sub</sub>. Moreover, a better correlation was observed when RE was scaled by allometric scaling rather than ratio scaling. In addition, females consistently displayed better RE than their male counterparts, regardless of whether RE was calculated from E<sub>c</sub> or VO<sub>2sub</sub>, scaled by ratio or allometry. Specifically, magnitude-based inferences revealed large differences when RE was expressed by ratio-scaled E<sub>c</sub> (ES = 1.55, RE was averaged across three intensities; ES = 1.66, 1.64, 1.23 for 55%, 65%, and 75%VO<sub>2max</sub>, respectively) and  $VO_{2sub}$  (ES = 1.51, RE was averaged across three intensities; ES = 1.61, 1.60, and 1.2 for 55%, 65%, and 75%VO<sub>2max</sub>, respectively), all with 100% possibility. Magnitude-based inferences also revealed extremely large differences when RE was expressed by allometric-scaled  $E_c$  (ES = 6.63, RE was averaged across three intensities;

Table 2. The coefficient of determination  $(R^2)$  from linear and allometric scaling between body weight and both  $E_c$  and  $VO_{2sub}$ .

Variable	Gender	Intensity -	$\mathbb{R}^2$	
v ariabie			Linear	Allometric
	Male	55%VO <sub>2max</sub>	0.162	0.192
		$65\%VO_{2max}$	0.153	0.169
		$75\%VO_{2max}$	0.115	0.136
		Overall	0.155	0.178
Ec (kcal/min)		55%VO <sub>2max</sub>	0.159	0.174
Ec (Kcai/IIIII)	Female	$65\%VO_{2max}$	0.194	0.204
	remaie	$75\%VO_{2max}$	0.301	0.314
		Overall	0.242	0.255
	Male	$55\%VO_{2max}$	0.155	0.184
		$65\%VO_{2max}$	0.148	0.166
		$75\%VO_{2max}$	0.106	0.128
		Overall	0.147	0.171
VO <sub>2sub</sub> (ml/min)		$55\%VO_{2max}$	0.156	0.171
	Female	65%VO <sub>2max</sub>	0.193	0.202
	remaie	$75\%VO_{2max}$	0.284	0.298
		Overall	0.233	0.246

Table 3. Correlations between RE expressions and body weight.

	Gender	RE expressions	Body weight (kg)		
Intensity			r	р	
		kcal/kg/min	-0.41	0.02	
	Male	ml/kg/min	-0.42	0.01	
		kcal/kg <sup>0.53</sup> /min	-0.04	0.81	
55%VO <sub>2max</sub>		ml/kg <sup>0.52</sup> /min	-0.04	0.81	
55% V U <sub>2max</sub>	Female	kcal/kg/min	-0.27	0.11	
		ml/kg/min	-0.26	0.13	
		kcal/kg <sup>0.63</sup> /min	-0.03	0.86	
		ml/kg <sup>0.63</sup> /min	-0.03	0.87	
	Male	kcal/kg/min	-0.47	0.01	
		ml/kg/min	-0.47	< 0.01	
	Maie	kcal/kg <sup>0.48</sup> /min	-0.05	0.77	
65%VO <sub>2max</sub>		ml/kg <sup>0.48</sup> /min	-0.05	0.77	
05 70 V O2max		kcal/kg/min	-0.24	0.16	
	Female	ml/kg/min	-0.23	0.18	
	remaie	kcal/kg <sup>0.67</sup> /min	-0.02	0.92	
		ml/kg <sup>0.68</sup> /min	-0.02	0.93	
		kcal/kg/min	-0.46	0.01	
	Male	ml/kg/min	-0.47	< 0.01	
		kcal/kg <sup>0.44</sup> /min	-0.04	0.82	
75%VO <sub>2max</sub>		ml/kg <sup>0.43</sup> /min	-0.04	0.81	
7570 V O2max	Female	kcal/kg/min	-0.18	0.32	
		ml/kg/min	-0.18	0.30	
		kcal/kg <sup>0.81</sup> /min	-0.03	0.88	
		ml/kg <sup>0.79</sup> /min	-0.03	0.89	
	Male	kcal/kg/min	-0.47	< 0.01	
		ml/kg/min	-0.47	< 0.01	
		kcal/kg <sup>0.48</sup> /min	-0.05	0.79	
Overall		ml/kg <sup>0.47</sup> /min	-0.05	0.79	
Overali	Female	kcal/kg/min	-0.24	0.17	
		ml/kg/min	-0.24	0.17	
		kcal/kg <sup>0.71</sup> /min	-0.03	0.88	
		ml/kg <sup>0.71</sup> /min	-0.03	0.88	

ES = 4.45, 6.17, and 7 for 55%, 65%, and 75%VO<sub>2max</sub>, respectively) and VO<sub>2sub</sub> (ES = 6.61, RE was averaged across three intensities; ES = 4.68, 6.17, and 6.91 for 55%, 65%, and 75%VO<sub>2max</sub>, respectively), all with 100% possibility.

#### **Discussion**

Previous studies have mainly focused on the application of ratio-scaled VO<sub>2sub</sub> (Hung et al., 2019). However, VO<sub>2sub</sub> cannot avoid the influence of substrate-related issues, and the application of ratio scaling has been met with skepticism. E<sub>c</sub> may be a more reliable measure, but previous studies exploring allometric scaling applied to E<sub>c</sub> are limited and lack verification of practical application (Shaw et al., 2014; Zakeri et al., 2006). Thus, this study aimed to explore: 1) the validity of VO<sub>2sub</sub> as a quantification of RE by comparing this variable with the underlying E<sub>c</sub> of running; 2) the appropriateness of ratio scaling in normalizing RE measures. No disparity was found between changes in VO<sub>2sub</sub> and E<sub>c</sub> as running intensity increased. A further finding was that allometric scaling, rather than ratio scaling, was more appropriate to account for body weight when using both measures to quantify RE. Moreover, RE expressed by allometric-scaled E<sub>c</sub> (kcal/kg<sup>b</sup>/min) showed a higher correlation with running performance.

#### Comparison among different RE measurements

It has been argued that VO<sub>2sub</sub>, as the quantification of RE,

may not accurately reflect the underlying energy cost since it ignores issues related to substrates (di Prampero et al., 2009; Fletcher et al., 2009; Jeukendrup and Wallis, 2005; Shaw et al., 2014). Instead, direct quantification of RE using  $E_c$  considers this influence and aligns with the definition of RE, making it a more accurate, meaningful measure. This assertion is, at least partly, supported by the consistently higher  $R^2$  values in the regression models (regardless of allometric scaling or linear function) when  $E_c$  was the dependent variable (Table 2).

However, other findings in this study do not entirely reject the validity of VO<sub>2sub</sub>. As indicated by the one-way repeated-measures ANOVA, E<sub>c</sub> (kcal/kg/min) increased progressively with rising running intensity. This trend is consistent with previous studies indicating that energy cost increases as exercise intensity rises (Beneke and Leithäuser, 2017; Fletcher et al., 2009; Shaw et al., 2014). As intensity increases, the demand for adenosine triphosphate (ATP) also rises, prompting a metabolic shift toward greater carbohydrate utilization. Although fat oxidation yields more energy per gram than carbohydrate oxidation (9.75 kcal vs. 4.07 kcal), the high efficiency of carbohydrates in producing "quicker" energy is more crucial in higher-intensity exercise that demands a greater amount of ATP, which in turn contributes to heightened oxygen consumption (Costill, 1988; Jeukendrup and Wallis, 2005).

Table 4. Correlations between different RE expressions and running performance.

T4	Gender	R		
Intensity		Expressions	Means±SD	r
	Male	kcal/kg/min	$0.16\pm0.02$	0.35*
		kcal/kg <sup>0.53</sup> /min	$1.16\pm0.13$	$0.43^{*}$
		ml/kg/min	$32.49\pm4.15$	$0.35^{*}$
55%VO <sub>2max</sub>		ml/kg <sup>0.52</sup> /min	$243.59\pm27.46$	$0.42^{*}$
33 70 V U2max	Female	kcal/kg/min	$0.13\pm0.02$	0.34*
		kcal/kg <sup>0.63</sup> /min	$0.56\pm0.09$	$0.35^{*}$
		ml/kg/min	$25.56\pm4.59$	0.33
		ml/kg <sup>0.63</sup> /min	$109.97 \pm 18.88$	$0.35^{*}$
	Male	kcal/kg/min	$0.19\pm0.02$	0.41*
		kcal/kg <sup>0.48</sup> /min	$1.63\pm0.17$	0.51**
		ml/kg/min	$37.57 \pm 4.63$	$0.41^{*}$
65%VO <sub>2max</sub>		ml/kg <sup>0.48</sup> /min	330.31±34.67	0.51**
03 70 V O2max	Female	kcal/kg/min	$0.15\pm0.02$	0.45**
		kcal/kg <sup>0.67</sup> /min	$0.55\pm0.09$	0.46**
		ml/kg/min	$29.87 \pm 5.10$	0.44**
		ml/kg <sup>0.68</sup> /min	107.99±17.91	0.45**
	Male	kcal/kg/min	$0.21\pm0.03$	$0.41^{*}$
		kcal/kg <sup>0.44</sup> /min	$2.16\pm0.25$	$0.50^{**}$
		ml/kg/min	$41.89\pm5.67$	$0.40^{*}$
75%VO <sub>2max</sub>		ml/kg <sup>0.43</sup> /min	453.96±52.19	0.50**
73 /0 V O2max	Female	kcal/kg/min	$0.17\pm0.03$	0.27
		kcal/kg <sup>0.81</sup> /min	$0.37\pm0.05$	0.27
		ml/kg/min	$34.83\pm5.28$	0.26
		ml/kg <sup>0.79</sup> /min	79.17±11.81	0.26
	Male Female	kcal/kg/min	$0.19\pm0.02$	$0.41^{*}$
		kcal/kg <sup>0.48</sup> /min	$1.64\pm0.17$	$0.51^{*}$
		ml/kg/min	$37.31\pm4.62$	$0.41^{*}$
Overall		ml/kg <sup>0.47</sup> /min	340.64±35.77	0.51*
Overall		kcal/kg/min	$0.15\pm0.02$	$0.37^{*}$
		kcal/kg <sup>0.71</sup> /min	$0.47\pm0.07$	$0.38^{*}$
		ml/kg/min	$30.09\pm4.74$	$0.36^{*}$
* n < 0.05: ** r		ml/kg <sup>0.71</sup> /min	95.04±14.52	$0.37^{*}$

\*, p < 0.05; \*\*, p < 0.01.

Our findings support this statement, as we observed a decrease in fat% and an increase in VO<sub>2sub</sub> with increasing exercise intensity. Overall, the observed increase in VO<sub>2sub</sub> (ml/kg/min) across running intensities in this study suggests that VO<sub>2sub</sub> remains a valid indirect measure of RE, at least within the context of our participant cohort. This finding aligns with the concurrent rise in E<sub>c</sub>, supporting the notion that VO<sub>2sub</sub> can reflect changes in metabolic demand during incremental exercise. However, this result contrasts with previous studies that reported a divergence between E<sub>c</sub> and VO<sub>2sub</sub>, where E<sub>c</sub> increased with intensity while VO<sub>2sub</sub> remained relatively stable VO<sub>2sub</sub> (Fletcher et al., 2009; Shaw et al., 2014). One plausible explanation is the difference in participant characteristics. Unlike earlier studies that focused on elite athletes, our study involved amateur runners, whose physiological responses and metabolic profiles may differ significantly (e.g., lower fat% in this study). Another possible explanation is that the conversion of the original unit, in which the speed information (km/h) was incorporated and the unit was changed from ml/kg/min to ml/kg/km (oxygen consumption per unit of distance), may have compromised its sensitivity to intensity. As a result, such conversion may lead to a misleading conclusion that RE was independent of speed changes (Fletcher et al., 2009; Shaw et al., 2014).

Although  $VO_{2sub}$  is a valid quantification of RE in this study, inter-individual comparisons in RE are valid

only when the statistical adjustment is reliable and accurate enough to remove the influence of body weight. According to the results shown in Table 2, the R<sup>2</sup> of allometric scaling (from 0.128 to 0.314) was consistently higher than that of the linear function (ranging from 0.106 to 0.301), regardless of whether E<sub>c</sub> or VO<sub>2sub</sub> was used. Additionally, regardless of Ec or VO<sub>2sub</sub>, the calculated exponent b ranged from 0.43 to 0.81, aligning closer to the 2/3 theory (Günther, 1975; Lee and Zhang, 2021). As early as 1838, the "surface law", which states that the basal metabolism of animals with different sizes is nearly proportional to their body surfaces, was recommended to describe the relationship between physiological function variables and body dimensions (Lee and Zhang, 2021). According to the law, metabolic rate should be proportional to body weight raised to the power of 2/3. Later, in 1927, Lambert and Teissier also confirmed this theoretical exponent b value for body weight from a new perspective—kinematic or biological similarity (KBS), which combined Newton's dimensional analysis with geometric similarity (Lee and Zhang, 2021). The KBS believes that the relationship between physiological function and body weight should satisfy the equation of " $x = w^{\alpha + \frac{1}{3}\beta + \frac{1}{3}\gamma}$ ", where x represents a physiological function and w represents body weight (Lee and Zhang, 2021). Given that  $\alpha=-1$ ,  $\beta=4$ , and  $\gamma=1$  in the relationship between oxygen consumption and body weight, the same result, "2/3", can be calculated (Lee and

Zhang, 2021). Although the other theoretical b value, "3/4", has been extrapolated to scale body weight, with some empirical studies on the relationship between VO<sub>2max</sub> and body weight supporting it, no consensus has yet been reached on which theoretical b value is better (Lee and Zhang, 2021; Lolli et al., 2017; West et al., 1997). However, (Darveau et al., 2002) believed that the increased importance of energy-supply processes and decreased reliance of energy-demand processes due to the shifts of metabolic rate from resting to maximal condition may cause a significant increase in b value. Moreover, some scholars believed that both 2/3 and 3/4 laws are applicable, but the 2/3 law might be more suitable for an intraspecific (withinspecies) mass exponent (Batterham et al., 1999). Thus, the 2/3 law appears to be more appropriate for application in scaling RE, and our findings, at least partly, support this law. Furthermore, the probabilities of these b values being less than 1 ranged from likely to most likely. These results indicate that the traditional ratio-scaled RE (ml/kg/min) is untenable. Instead, allometric scaling with a higher R<sup>2</sup> should be a more accurate statistical adjustment for RE measurement. Further analysis of the 'removal effect of body weight' (Table 3) supports this viewpoint, as no correlations with body weight were observed when allometric scaling, rather than ratio scaling, was applied. This indicates that RE expressed by allometric scaling can effectively eliminate the influence of body weight, whereas the linear function is not powerful enough to exclude that influence. (Chamari et al., 2005) stated that the RE of senior soccer players might be overestimated when expressed in the traditional way (ml/kg/min). Inappropriate statistical adjustments may lead to faulty information, thereby misleading coaches' training schedules.

When comparing allometric-scaled  $E_c$  and allometric-scaled  $VO_{2sub}$ , the former consistently showed a better  $R^2$  (Table 2). Combined with the results of the 'removal effect of body weight', allometric-scaled  $E_c$  can better improve the accuracy of RE compared to allometric-scaled  $VO_{2sub}$ . Overall, although  $VO_{2sub}$  is a valid quantification of RE in this study, consistently higher  $R^2$  values in regression models with  $E_c$  as the dependent variable suggest  $E_c$  to be a more appropriate quantification of RE. Furthermore, allometric scaling, rather than ratio scaling, should be applied to normalize these quantifications in inter-individual comparisons. For the convenience of calculation, the 2/3 law can be considered for the body weight exponent.

#### RE and its correlations with performance

Due to the unreliable nature of RE reflected by the ratioscaled  $VO_{2sub}$ , it is recommended to reconsider findings and conclusions to avoid providing misleading feedback or information to coaches or scholars (e.g., overestimating one's real RE, inappropriate training arrangements).

RE is a controversial topic in terms of gender. Some studies have found that males have better RE than females, while others have argued that females have better or that no gender difference exists (Ariëns et al., 1997; Daniels and Daniels, 1992; Davies et al., 1997; Helgerud, 1994; Janz et al., 1998). A key factor contributing to these inconsistent findings is the measure of RE. In this study, we expressed RE with allometric-scaled E<sub>c</sub> and observed similar

results to findings by (Helgerud, 1994), suggesting that males' RE is not better than females' RE. This is also supported by the relatively higher RER (Table 1) and lower fat% in males compared to females, suggesting that males relied more on carbohydrates as an energy source. Magnitude-based inferences suggest that the likelihood of future similar studies observing these extremely large gender differences in RE is most likely. Although a definitive conclusion cannot be made based on the limited evidence, the uncertainty of RE in gender is unlikely due to the sample's sports background. Specifically, when RE was scaled by ratio-scaled VO<sub>2sub</sub>, no gender difference was observed in a study by (Helgerud, 1994) with intermediate national standard elite athletes, or by (G et al., 2020) with amateur runners similar to our study. Therefore, while ratio-scaled VO<sub>2sub</sub> in the current study also successfully detected large differences in RE between females and males, the mathematical limitations of ratio scaling raise concerns about the validity of such findings. Based on these considerations, we speculated that statistical defects of ratio scaling in normalizing RE are the critical reason for controversy concerning gender differences in RE, contributing to misleading information. More studies based on allometric scaling are needed before making a potent conclusion regarding the gender difference in RE.

To further evaluate the ability of allometric-scaled E<sub>c</sub> in explaining performance, we applied different expressions of RE in practice. Correlation analyses revealed that RE scaled by allometric E<sub>c</sub> consistently displayed a better correlation with running performance and was more sensitive in detecting correlations at increasing intensities. Notably, these correlations remained evident in male participants but disappeared in females at 75%VO<sub>2max</sub>. This finding raises important considerations regarding the appropriateness of exercise intensity when assessing RE. Normally, submaximal intensity in RE should not exceed 85% VO<sub>2max</sub> (Williams et al., 1991) to ensure a stable physiological state. However, this recommendation was primarily based on data from male athletes with relatively high aerobic capacities and may not be applicable to amateur populations, especially female runners. In our study, elevated blood lactate levels and RER observed in females at 75% VO<sub>2max</sub> (see Table 1) suggest a deviation from steady-state conditions. The elevated intensity may induce greater neuromuscular demand and activation of type II fibers (Hargreaves and Spriet, 2020), which may help to cope with this high exercise intensity anaerobically and result in an unstable state (i.e., a small increase in oxygen uptake). Therefore, the sensitivity and accuracy of RE scaled by allometric E<sub>c</sub> may be compromised, thereby weakening the observed correlations with performance. However, the anaerobic contribution to ATP production should be limited, as only approximately 15% of ATP is attributed to anaerobic contribution during a 6-minute maximal exercise (Gastin, 2001). Overall, the recommended intensity for the RE test limited at around 65%VO<sub>2max</sub> seems to be more appropriate, as the highest correlations were observed around this intensity.

#### Limitations

The present study has several limitations. First, additional

activities may influence athletic performance. Although participants' exercise frequency and intensity were self-reported, we did not strictly monitor their daily physical activities using validated pedometers. Second, this study only included recreationally active male and female college students. Therefore, the findings should be limited to this population. Meanwhile, the sample size of this study is still small. To ensure the applicability, reliability, and accuracy of RE measured by allometric-scaled E<sub>c</sub>, further studies should increase the sample size and focus on subjects with different characteristics or backgrounds (e.g., athletes). Third, it was challenging to assess whether participants exerted their best to complete the 1000-meter field test, despite arranging a group of participants in the test to simulate the tournaments and providing them with strong encouragement. Moreover, since it was impossible for participants to complete all the laboratory tests on the same day, the 1000-meter outdoor performance took place on different days. This may influence the validity of the performance because multiple confounding factors, including wind speed, surface moisture and friction, drafting of other participants, and pacing familiarity, may influence the performance. Future studies may consider an indoor performance test to better control these factors. Fourth, the menstrual cycle was not strictly controlled at the same phase (e.g., luteal phase) for female participants, although tests were rescheduled to avoid periods when female participants were menstruating. Finally, some individuals experienced relatively higher blood lactate (i.e., blood lactate greater than the commonly used reference point for lactate threshold at 4 mmol/L) and RER during the RE tests, which may indicate the 'false' steady state. Although the small proportion of them (~10%) may not greatly influence the power of our findings, future studies should consider improving the protocols, including extending the break time before each test, monitoring baseline blood lactate, and evaluating the specific lactate threshold for each participant.

#### Conclusion

For the convenience of application,  $VO_{2sub}$  can be a valid quantification of RE. However, its application should be restricted to individuals who share characteristics similar to those in this study (i.e., amateur runners). In contrast,  $E_c$ , as the quantification of RE, is recommended for studies seeking accuracy, as it aligns with the definition of RE and can reflect the underlying energy cost during submaximal intensity running.  $65\%VO_{2max}$  intensity as the submaximal testing intensity in the RE test is recommended. Furthermore, allometric scaling, rather than ratio scaling, is more appropriate to normalize RE quantifications, as it can effectively remove the influence of body weight in inter-individual comparisons. For the convenience of allometric calculation, directly applying the 2/3 law in allometric scaling seems acceptable.

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#### **Key points**

- While the widely used submaximal oxygen uptake (VO<sub>2sub</sub>) does not directly capture metabolic consumption during exercise, it is a valid alternative to energy cost (E<sub>c</sub>) used to quantify running economy (RE).
- Allometric scaling, rather than ratio scaling, is a more appropriate statistical adjustment for normalizing RE quantifications in interindividual comparisons.
- For studies seeking high precision, allometric-scaled E<sub>c</sub>, which provides a more accurate and practical measure of RE, is recommended to quantify RE.
- For the convenience of allometric calculation, directly applying the 2/3 law in allometric scaling seems acceptable.

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