# Physiological aspects and energetic contribution in 20s:10s high-intensity interval exercise at different intensities 

Gabriel V. Protzen ${ }^{1}$, Charles Bartel ${ }^{1,4}$, Victor S. Coswig ${ }^{2}$, Paulo Gentil ${ }^{3}$ and Fabricio B. Del Vecchio ${ }^{1}$<br>${ }^{1}$ Physical Education College, Federal University of Pelotas, Pelotas, Rio Grande do Sul, Brazil<br>${ }^{2}$ Faculty of Physical Education, Federal University of Para, Castanhal, Pará, Brazil<br>${ }^{3}$ Faculty of Physical Education and Dance, Federal University of Goias, Goiânia, Goiás,<br>${ }^{4}$ Physical Education Center Admiral Adalberto Nunes, Brazilian Navy, Rio de Janeiro, Brazil

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Corresponding author
Gabriel V. Protzen, gprotzen@gmail.com

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#### Abstract

Background. One of the most popular high-intensity interval exercises is the called "Tabata Protocol". However, most investigations have limitations in describing the work intensity, and this fact appears to be due to the protocol unfeasibility. Furthermore, the physiological demands and energetic contribution during this kind of exercise remain unclear. Methods. Eight physically active students ( $21.8 \pm 3.7$ years) and eight well-trained cycling athletes ( $27.8 \pm 6.4$ years) were enrolled. In the first visit, we collected descriptive data and the peak power output (PPO). On the next three visits, in random order, participants performed interval training with the same time structure (effort:rest $20 \mathrm{~s}: 10 \mathrm{~s}$ ) but using different intensities ( $115 \%, 130 \%$, and $170 \%$ of PPO). We collected the number of sprints, power output, oxygen consumption, blood lactate, and heart rate. Results. The analysis of variance for multivariate test (number of sprints, power output, blood lactate, peak heart rate and percentage of maximal heart rate) showed significant differences between groups ( $F=9.62$; $p=0.001$ ) and intensities ( $F=384.05$; $p<$ 0.001 ), with no interactions ( $F=0.94 ; p=0.57$ ). All three energetic contributions and intensities were different between protocols. The higher contribution was aerobic, followed by alactic and lactic. The aerobic contribution was higher at $115 \%$ PPO, while the alactic system showed higher contribution at $130 \%$ PPO. In conclusion, the aerobic system was predominant in the three exercise protocols, and we observed a higher contribution at lower intensities.


Subjects Biochemistry, Anatomy and Physiology, Metabolic Sciences
Keywords High-intensity interval exercise, High-intensity interval training, Energy system contribution, Physiological aspects, Anaerobic capacity, Tabata protocol

## INTRODUCTION

High-intensity interval exercise (HIIE) is repeated efforts with intensity above $90 \%$ of the intensity related to maximal oxygen consumption (iV. $\mathrm{O}_{2 \mathrm{MAX}}$ ) followed by active or passive recovery (Buchheit \& Laursen, 2013b; MacInnis \& Gibala, 2017). Effort and recovery duration and intensity are the mainly manipulated variables during HIIE, which distinctly
affect acute and chronic metabolic responses (MacInnis \& Gibala, 2017). However, the inconsistency and variability in HIIE protocols may limit its external validity and the data extrapolation to different populations (Viana et al., 2018b).

One of the most popular HIIE structure (Tabata, 2019) was proposed by Tabata et al., (1996), which is also one of the most inconsistently applied protocols (Gentil et al., 2016). It is seven to eight repetitions of 20 s of effort and 10 s of passive recovery ( $20 \mathrm{~s}: 10 \mathrm{~s}$ ) performed until the participant was unable to keep at least 85 rpm . This HIIE model is at an intensity equivalent to 1.7 times the measured $\mathrm{V} \cdot \mathrm{O}_{2 \mathrm{MAX}}$, which was calculated by the extrapolation of the linear relationship between submaximal exercise intensity and oxygen uptake ( $7-8 \mathrm{x}$ 20s @170V• $\mathrm{O}_{2 \mathrm{MAX}}$ : 10s of passive recovery). Previously, in this type of HIIE, acute studies observed high oxygen consumption (Viana et al., 2018c), elevated glycolysis, pronounced glycogen depletion (Scribbans et al., 2014), and high parasympathetic inhibition (Schaun $\leftrightarrow$ Del Vecchio, 2018). Intermittent efforts at $170 \%$ of iV• $\mathrm{O}_{2 \mathrm{MAX}}$, obtained with a graded exercise test, are indicated for sprint interval training or repeated sprint training, but not for short HIIE (Buchheit \& Laursen, 2013b). In a study carried out with physically active young men on magnetic bikes, Viana et al. (2018c) verified that the $170 \%$ of $\mathrm{iV} \cdot \mathrm{O}_{2 \text { max }}$ intensity allowed an average of only four repetitions, and induced a very short period at high oxygen consumption rates. However, to date, the effect of this type of exercise in highly trained cycling athletes habituated to this exercise model is unknown.

Notwithstanding, HIIE models based on Tabata et al. (1996) have been widely used to improve the metabolic profile or increase physical fitness (Bonafiglia et al., 2017; Domaradzki et al., 2020; Logan et al., 2016; Ma et al., 2013; McRae et al., 2012; Scribbans et al., 2014). However, many investigations (Domaradzki et al., 2020; Logan et al., 2016; Ma et al., 2013; Scribbans et al., 2014) have limitations in describing effort intensity, using all-out efforts or different from the intensity corresponding to $170 \%$ of iV. $\mathrm{O}_{2 \mathrm{MAX}}$, therefore, differs from the original protocol (Tabata et al., 1996; Viana et al., 2018a).

Regarding energetic responses, the authors claimed that the 20s:10s protocol reached maximal aerobic and anaerobic demands (Tabata, 2019; Tabata et al., 1996). This statement was because, at the end of the protocol, the subjects reached their maximal accumulated oxygen deficit (MAOD) and an oxygen uptake equal to their $\mathrm{V} \cdot \mathrm{O}_{2 \mathrm{MAX}}$. However, the model applied has been previously questioned (Bangsbo, 1992), and the acute physiological impact of different intensities in 20s:10s HIIE on cardiorespiratory and neuromuscular variables is unknown. Information about the contribution of energetic systems during the 20 s:10s protocol is essential to understand its physiological demands, and to date, we are aware of no studies have analysed such responses.

Therefore, considering that this knowledge is relevant to the exercise organisation, as it allows to drive physiological stimuli according to the training status, the objectives of the present study were to measure the physiological demands (oxygen uptake, heart rate, and blood lactate), to assess the contribution of energy systems, as well as neuromuscular parameters (total sprint number, mean, and maximum power output) in the 20s:10s HIIE protocol in three different intensities, in cycling athletes and non-athletes.

## MATERIALS \& METHODS

## Experimental approach to the problem

The study involved four visits, with minimum rest of 48 h and a maximum of 72 h between them. Participants were instructed not to ingest caffeine or alcohol and not practice intense physical exercises in the 48 h before each trial. On the first day, the ethical research aspects, body mass measurement (Soehnle®, Backnang, Denmark), and height (Standard, Sanny®, São Paulo, Brazil) were collected. On the same day, participants completed an incremental maximal effort test to identify: (i) maximal oxygen consumption (V. $\mathrm{O}_{2 \mathrm{MAX}}$ ); (ii) maximum heart rate $\left(\mathrm{HR}_{\mathrm{MAX}}\right)$; (iii) power associated with the maximum oxygen consumption ( $\mathrm{pV} \cdot \mathrm{O}_{2 \mathrm{MAX}}$ ) for sample characterisation and determination of training load.

In the following days, the participants performed the HIIE training sessions in three different intensities in a random order, separated by a minimum and maximum interval of 48 to 72 h , respectively. Subjects performed the training in a mechanical braking cycle ergometer (Biotec 2100, Cefise®, São Paulo, Brazil). Specific software was used to calculate the power output based on wheel speed and previously informed load (Ergometric 6.0, Cefise $\circledR$ ®, São Paulo, Brazil). The sessions were conducted by previously trained researchers, following the institutional safety manual.

## Subjects

We based the sample size calculation on data from Lopes-Silva et al. (2015). The authors observed that the mean difference for aerobic contribution during HIIE between the two conditions (placebo or substance) was $3 \%$, with standard deviation from means of $2.5 \%$. Seven individuals per group would be required, when assuming $80 \%$ power and $5 \%$ significance level in a two-tailed test. Considering sample loss of $10 \%$, eight individuals participated per group. The inclusion criteria were: (i) to be physically active (more than 150 min of physical activity per week); (ii) have no musculoskeletal, respiratory, or cardiovascular problems; (iii) non-smoker; (iv) declare not to be anabolic androgenic steroids user. Besides, cyclists should: (i) practice road cycling or mountain bike marathon for more than two years and (ii) have a relative $\mathrm{V} \cdot \mathrm{O}_{2 \mathrm{MAX}}$ equal to or greater than 50 $\mathrm{mL} . \mathrm{kg}^{-1} \cdot \mathrm{~min}^{-1}$. The sample consisted of 16 males, of whom eight sports-engaged physical education students (age: $21.8 \pm 3.7$; body mass: $65.5 \pm 5.4 \mathrm{~kg} ; \mathrm{HR}_{\mathrm{MAX}}: 192.7 \pm 2.6$ bpm; V. $\left.\mathrm{O}_{2 \mathrm{MAX}}: 3375.5 \pm 331.4 \mathrm{mLO}_{2} \cdot \mathrm{~min}^{-1} ; \mathrm{pV} \cdot \mathrm{O}_{2 \mathrm{MAX}}: 238.1 \pm 18.0 \mathrm{~W}\right)$ and eight road cycling or mountain-bike athletes (age: $27.8 \pm 6.4$; body mass: $70.3 \pm 9.5 \mathrm{~kg} ; \mathrm{HR}_{\mathrm{MAX}}$ : $\left.188.4 \pm 4.5 \mathrm{bpm} ; \mathrm{V} \cdot \mathrm{O}_{2 \mathrm{MAX}}: 4122.2 \pm 506.7 \mathrm{mLO}_{2} \cdot \mathrm{~min}^{-1} ; \mathrm{pV} \cdot \mathrm{O}_{2 \mathrm{MAX}}: 351.8 \pm 42.6 \mathrm{~W}\right)$. All cyclists reported doing at least eight hours of weekly training. All subjects filled informed consent, and the Federal University of Pelotas Research Ethical Committee approved the research project (protocol number 77729517.1.0000.5313).

## Incremental maximal effort test

The incremental test started with a 2 -min continuous warm-up with 0.5 kgf load. After warming up, we increased the load every minute by 0.25 kgf , which corresponds to approximately 25 w , until the participant reached exhaustion, or was not able to maintain the minimum cadence of 90 rpm .

## Training protocols

The experimental sessions began with a 5 -min warm-up using a 0.5 kgf load with a cadence between 90 and 100 rpm .

The training protocol followed the previously published pattern of effort:pause of the 20s:10s proposed by Tabata et al. (1996), and was performed using the following intensities: (i) $115 \%$; (ii) $130 \%$; (iii) $170 \%$ of iV. $\mathrm{O}_{2 \mathrm{MAX}}$ (denominated $115 \% \mathrm{PPO}, 130 \% \mathrm{PPO}$, and $170 \%$ PPO, respectively), with a cadence control between 90 and 100 rpm . The subjects were oriented to perform as many sprints as possible and to remain seated on the bicycle. The handlebar and saddle were individually adjusted, and we used the same settings in all experimental sessions. The training was interrupted when the subject could not maintain the minimum predefined cadence of 90 rpm or declared voluntary exhaustion.

## Gas exchanges collection

The $\mathrm{V} \cdot \mathrm{O}_{2}$ was estimated at every three breaths using an open-circuit gas analyser (V.O2000 ${ }^{\mathrm{TM}}$, Medical Graphics, Minnesota, US), previously calibrated and following the manufacturer's guidelines. In order to collect gas exchange, a Neoprene ${ }^{\mathrm{TM}}$ mask with a high flow pneumotachograph was connected by an umbilical to V.O2000.

Gas exchanges were recorded with the participant sitting on the cycle ergometer for 5 min (Campos et al., 2012), to measure oxygen consumption associated with relative rest before the maximal incremental test. This monitoring also occurred during the test to identify the $\mathrm{V} \cdot \mathrm{O}_{2 \mathrm{MAX}}$, which was considered as the mean of last-minute values. During training, were collected $\mathrm{V} \cdot \mathrm{O}_{2}$ data in three different moments in each exercise protocols: (i) relative rest, (ii) during the whole activity, and (iii) after the end of the exercise, for seven minutes (Brooks \& Mercier, 1994).

## Blood lactate analysis

A sample of $15 \mu \mathrm{~L}$ of blood was obtained from the finger, drained to a heparinised capillary, and transferred to a microtube (Eppendorf ${ }^{\mathrm{TM}}$ ) with $30 \mu \mathrm{~L}$ of EDTA anticoagulant to measure the blood lactate concentration ( $\left[\mathrm{La}^{-}\right]$). We performed the analysis on a lactate analyser (YSI $2300^{\text {TM }}$ Stat Plus, Yellow Springs, Ohio, US). Blood was collected in relative rest and minutes $1,3,5$, and 7 after the end of each training protocols, in order to obtain the peak lactate concentration ( $\left[\mathrm{La}^{-}\right]_{\text {PEAK }}$ ).

## Heart rate data collection

The heart rate was measured by a specific monitor (V800 ${ }^{\mathrm{TM}}$, Polar Electro, Kempele, FI), previously validated (Giles, Draper e Neil, 2016). Participants wore a thoracic strip with a cardiac sensor and data recorded in the watch. These procedures were used during the incremental test to obtain maximal heart rate $\left(\mathrm{HR}_{\mathrm{MAX}}\right)$ and during each trial $(115 \% \mathrm{PPO}$, $130 \% \mathrm{PPO}$, and $170 \% \mathrm{PPO}$ ) to obtain the peak heart rate ( $\mathrm{HR}_{\text {PEAK }}$ ) in order to characterise the cardiovascular requirements of an exercise model.

## Calculation of energy system contributions

We presented the energy contribution in relative (\%) and absolute (kilocalories and kilojoules) values. We assumed that one litre of oxygen is calorically equivalent to 20.9
kilojoules and 5 kilocalories. Aerobic energy was estimated using the $\mathrm{V} \cdot \mathrm{O}_{2}$ during exercise protocols subtracted by the $\mathrm{V} \cdot \mathrm{O}_{2 \text { rest }}$ through the trapezoidal method (Bertuzzi et al., 2007). The $\mathrm{V} \cdot \mathrm{O}_{2 \text { rest }}$ was obtained five minutes before the beginning of the protocol, with the subjects seated. The difference between the $\left[\mathrm{La}^{-}\right]_{\text {peak }}$ and the $\left[\mathrm{La}^{-}\right]_{\text {rest }}$ was used in the equation to estimate the energy production from the anaerobic lactic system, assuming that the accumulation of $1 \mathrm{mmol} . \mathrm{L}^{-1}$ is equivalent to $3 \mathrm{ml} . \mathrm{O}_{2} \cdot \mathrm{~kg}^{-1}$ of body mass (Di Prampero \& Ferretti, 1999; Margaria et al., 1963). The fast component of the excess post-exercise oxygen consumption $\left(\mathrm{EPOC}_{\text {fast }}\right)$, which is similar to maximal accumulated oxygen deficit (Zagatto et al., 2019), was used to estimate the production of alactic energy (Di Prampero \& Ferretti, 1999; Haseler, Hogan \& Richardson, 1999). We observed that the slow component of the bi-exponential model was insignificant. Therefore, we used the monoexponential model (Eq. (1)), and the estimation was calculated by the integration of the exponential part (Eq. (2)). These procedures were applied in previous research (Bertuzzi et al., 2007; Campos et al., 2012) and follow previously described assumptions (Di Prampero \& Ferretti, 1999). We used specific software (GEDAE-LaB, São Paulo, Brazil) to calculate the energetic contributions. The procedure was tested and validated against traditional calculations, with an intraclass correlation coefficient of 0.94 for energy expenditure and energy contribution calculation (Bertuzzi et al., 2016). Following, we provided the equations used by the software and their respective description; according to Bertuzzi et al. (2016).
$\mathrm{V} \cdot \mathrm{O}_{2(\mathrm{t})}=\mathrm{V} \cdot \mathrm{O}_{2 \text { rest }}+A\left[e^{-(t / t)}\right]$
$A L_{\text {ENERGY }}=A . \tau$
where $A L_{\text {ENERGY }}$ is alactic system contribution estimated by the fast component of excess post-exercise oxygen consumption, $\mathrm{V} \cdot \mathrm{O}_{2(\mathrm{t})}$ is the oxygen uptake at time $\mathrm{t}, \mathrm{V} \cdot \mathrm{O}_{2 \text { rest }}$ is the rest oxygen uptake, $A$ is the amplitude, and $t$ is the time constant.

## Statistical analysis

A Shapiro-Wilk test confirmed the data normality distribution, and we present data as mean and standard deviation (SD). Independent t -tests compared subjects' physical characteristics. We compared training protocols, energy systems, and power output with a two-way analysis of variance (variable $\times$ group) with repeated measures. We tested the sphericity of the data by Mauchly's test, and, when violated, applied Greenhouse-Geisser correction. Bonferroni posthoc identified the significant differences in power output, and Scheffé posthoc determined the differences between training protocols and energy systems.

## RESULTS

Independent t -tests indicated that athletes had lower maximal heart rate ( $p=0.04$ ) and higher absolute oxygen uptake ( $p=0.02$ ), age ( $p=0.04$ ), and peak power output ( $p<0.001$ ) than non-athletes.

The analysis of variance for the multivariate test (number of sprints, power output, blood lactate, peak heart rate, and percentage of maximal heart rate) resulted in significant differences between groups ( $F=9.62 ; p=0.001$ ) and intensities ( $F=384.05 ; p<0.001$ ),

Table 1 Descriptive data from mechanical and physiological variables ( $n=16$ ).

| Variable | Non-athletes$(n=8)$ |  | Athletes ( $n=8$ ) |  | Total |  | Group$\mathbf{F}(\mathbf{p})$ | Intensity$\mathbf{F}(\mathbf{p})$ | Interaction$\mathbf{F}(\mathbf{p})$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | Mean | $\pm$ SD | Mean | $\pm$ SD | Mean | $\pm$ SD |  |  |  |
| Number of sprints (reps)* |  |  |  |  |  |  | 1.2 (0.28) | 173.3 (<0.001) | 0.2 (0.79) |
| $115 \%$ PPO | 17.13 | $\pm 3.60$ | 15.50 | $\pm 3.34$ | 16.31 | $\pm 3.46$ |  |  |  |
| $130 \%$ PPO | 9.13 | $\pm 2.53$ | 8.13 | $\pm 1.81$ | 8.63 | $\pm 2.19$ |  |  |  |
| $170 \%$ PPO | 4.63 | $\pm 1.60$ | 3.88 | $\pm 1.25$ | 4.25 | $\pm 1.44$ |  |  |  |
| Mean power output (w)* |  |  |  |  |  |  | 18.2 (0.001) | 104.5 (<0.001) | 4.0 (0.65) |
| $115 \%$ PPO | 339.75 | $\pm 28.69$ | 411.25 | $\pm 52.21$ | 375.50 | $\pm 54.95$ |  |  |  |
| $130 \%$ PPO | 392.88 | $\pm 44.05$ | 515.38 | $\pm 60.18$ | 454.13 | $\pm 81.22$ |  |  |  |
| $170 \% \mathrm{PPO}$ | 503.00 | $\pm 50.50$ | 653.38 | $\pm 110.10$ | 578.19 | $\pm 113.48$ |  |  |  |
| Blood lactate (mmol.L ${ }^{-1}$ ) |  |  |  |  |  |  | 13.9 (0.002) | 4.46 (0.021) | 1.9 (0.16) |
| $115 \%$ PPO | 12.07 | $\pm 0.82$ | 14.37 | $\pm 1.53$ | 13.22 | $\pm 1.68$ |  |  |  |
| $130 \%$ PPO | 12.99 | $\pm 1.53$ | 13.66 | $\pm 0.88$ | 13.32 | $\pm 1.25$ |  |  |  |
| $170 \% \mathrm{PPO}$ | 11.28 | $\pm 1.60$ | 13.07 | $\pm 1.21$ | 12.17 | $\pm 1.65$ |  |  |  |
| Peak heart rate (bpm) ${ }^{\text {\# }}$ |  |  |  |  |  |  | 2.3 (0.15) | 309.6 (<0.001) | 0.3 (0.74) |
| $115 \%$ PPO | 180.88 | $\pm 9.19$ | 187.50 | $\pm 8.72$ | 184.19 | $\pm 9.30$ |  |  |  |
| $130 \%$ PPO | 180.63 | $\pm 9.78$ | 185.25 | $\pm 9.15$ | 182.94 | $\pm 9.45$ |  |  |  |
| $170 \%$ PPO | 174.13 | $\pm 12.32$ | 178.00 | $\pm 10.45$ | 176.06 | $\pm 11.22$ |  |  |  |
| Heart rate (\%HRmax) |  |  |  |  |  |  | 1.5 (0.24) | $162.2(<0.001)$ | 0.02 (0.98) |
| $115 \%$ PPO | 93.88 | $\pm 4.63$ | 99.49 | $\pm 4.02$ | 96.68 | $\pm 5.09$ |  |  |  |
| $130 \%$ PPO | 93.76 | $\pm 5.28$ | 98.28 | $\pm 4.17$ | 96.02 | $\pm 5.16$ |  |  |  |
| $170 \%$ PPO | 90.37 | $\pm 6.27$ | 94.41 | $\pm 4.34$ | 92.39 | $\pm 5.61$ |  |  |  |

Notes.
*all intensities are different between them $(p<0.001)$
\#difference between $115 \%$ and $170 \%(p<0.001)$
with no interactions. The results of univariate tests indicated that the number of sprints and peak heart rate were higher in lower intensities, while peak power output was higher at higher intensities (Table 1). The mean duration of each protocol was: 488, 258 and 127 s , for $115 \% \mathrm{PPO}, 130 \% \mathrm{PPO}$, and $170 \% \mathrm{PPO}$, respectively. Besides, athletes reached higher levels of blood lactate concentration than non-athletes.

Considering absolute contribution (Fig. 1C), there were significant differences between energetic systems ( $F=20.86 ; p<0.001$ ) and intensities ( $F=12.65 ; p=0.001$ ), with no interactions between systems and groups, intensities and groups, nor systems $\times$ intensities $\times$ groups. Pairwise comparisons using Bonferroni posthoc test localised differences between the three energetic systems, with a $p$-value lower than 0.01 , as well as between the three intensities, with $p$-values lower than 0.001 . Repeated measures showed a linear trend for decrease the participation of energetic system contribution (aerobic, lactic, and alactic) for $\mathrm{kcal}(F=98.49 ; p<0.001), \mathrm{kJ}(F=98.48 ; p<0.001)$ and litres of $\mathrm{O}_{2}(F=98.45$; $p<0.001$ ), as well as a linear reduction of kcal amount ( $F=110.2 ; p<0.001$ ), kJ amount ( $F=110.22 ; p<0.001$ ) and litres of $\mathrm{O}_{2}$ consumed ( $F=109.87 ; p<0.001$ ) considering all intensities ( $115 \%, 130 \%$, and $170 \%$ ). For the total amount of kcal, kJ , and litres of $\mathrm{O}_{2}$, no differences were found between non-athletes and athletes, but there were significant


Figure 1 Energetic system contribution during the 20 s: 10 s protocol at different intensitities ( $n=16$ ). (A) The relative contribution of the energetic system in all subjects. (B) Comparison between athletes and non-athletes of the relative contribution of the energetic system. (C) The absolute contribution of the energetic systems in non-athletes, athletes, and all subjects. * $=$ different from lactic contribution; \# = different from alactic contribution; $\mathrm{a}, \mathrm{b}, \mathrm{c}=$ different from $115 \% \mathrm{PPO}, 130 \% \mathrm{PPO}$, and $170 \%$ PPO respectively, considering each energetic system; $\dagger=$ different from cycling athletes, for the same intensity and energetic system; $\mathrm{PPO}=$ peak power output.

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differences between intensities ( $F=22.81 ; p<0.001$ ), with no interactions between groups and intensity. As a partial analysis by each energetic system, in total amount were found a linear trend for intensity in $\operatorname{kcal}(F=106.52 ; p<0.001), \mathrm{kJ}(F=106.52 ; p<0.001)$ and litres of $\mathrm{O}_{2}(F=109.9 ; p<0.001)$, with $p$-values lower than 0.001 for all comparisons ( $115 \% \mathrm{PPO}$ vs $130 \% \mathrm{PPO} ; 115 \% \mathrm{PPO}$ vs $170 \% \mathrm{PPO}$ and $130 \% \mathrm{PPO}$ vs $170 \% \mathrm{PPO}$ ).

Concerning relative energetic contribution system, there was no effect of group (nonathletes vs cycling athletes), but there were significant differences between intensities ( $F=39.3 ; p<0.001$ ), and systems ( $F=411.0 ; p<0.001$ ), with no significant interactions between group and intensity, group and energetic system. There was no interaction between intensity and energetic systems contribution ( $F=47.81 ; p<0.001$ ). Finally, we found no interaction between group, intensity, and energetic system.

Considering the intensity and energetic systems (Fig. 1A), at 115\%PPO and 130\%PPO, the aerobic contribution was higher than lactic $(p<0.001)$ and alactic ( $p<0.001$ ), with no difference between lactic and alactic. At $170 \% \mathrm{PPO}$, the aerobic contribution was different from lactic ( $p<0.001$ ) and alactic ( $p=0.02$ ), and lactic was different from alactic ( $p=0.04$ ). Additionally, the aerobic contribution was different considering the three selected intensities, $115 \% \mathrm{PPO}$ was higher than both $130 \% \mathrm{PPO}(p<0.001)$ and $170 \% \mathrm{PPO}$ ( $p<0.001$ ), and $130 \% \mathrm{PPO}$ was higher than $170 \% \mathrm{PPO}$ ( $p<0.001$ ). The lactic contribution was higher at $170 \% \mathrm{PPO}$ in comparison to $115 \% \mathrm{PPO}$, and alactic contribution was higher at $170 \% \mathrm{PPO}$ in comparison to $115 \% \mathrm{PPO}(p<0.001)$ and $130 \% \mathrm{PPO}(p=0.02)$.

In absolute values (Fig. 2A), we found significant differences for intensity ( $F=8.35$; $p=0.05$ ), with increased $\mathrm{O}_{2}$ consumption at $130 \% \mathrm{PPO}$ ( $p$-value $=0.005$ in comparison to $115 \% \mathrm{PPO}$ and $170 \% \mathrm{PPO}$ ), and higher $\mathrm{O}_{2}$ consumption in athletes ( $F=6.20 ; p=0.02$ ), with no interactions between intensities and groups $(F=4.36 ; p=0.05)$. Figure 2B shows that the three intensities reached different relative values to $\mathrm{V} \cdot \mathrm{O}_{2 \operatorname{MAX}}(F=3.25 ; p=0.05)$, and the post-hoc test pointed difference between $115 \% \mathrm{PPO}$ and $130 \% \mathrm{PPO}(p=0.008)$, with no differences between non-athletes and athletes, nor interactions. Additionally, there was a quadratic trend in these relative values ( $F=11.30 ; p=0.005$ ).

## DISCUSSION

The present study aimed to analyse and compare the energetic system contribution in the 20 s :10s HIIE with three different intensities in cycling athletes and non-athletes. As main findings, we found: (i) the relative dominance of the aerobic system in all three intensities when compared with lactic and alactic systems; (ii) the inability to perform the initially proposed number of sprints associated with $170 \% \mathrm{PPO}$ when the load is calculated from graded exercise test; (iii) the $130 \%$ PPO promoted higher oxygen consumption, but only in cyclists.

Previously, Gaitanos et al. (1993) showed that the lactic system participation reduced significantly from the first to the tenth sprint of $6 \mathrm{~s}: 30 \mathrm{~s}$, and, at the end of the exercise, the energy was predominantly from the alactic system, followed by an increase in aerobic contribution. Trump et al. (1996) applied intermittent exercise with different effort:pause structure (30s:240s). However, the results have a similar reduction in anaerobic systems


Figure 2 Oxygen consumption during the 20s:10s exercise at different intensities ( $\boldsymbol{n}=\mathbf{1 6}$ ). (A) Relative to body mass and (B) relative to the maximal oxygen consumption, at $115 \% \mathrm{PPO}, 130 \% \mathrm{PPO}$, and $170 \% \mathrm{PPO}$, in non-athletes and athletes. $\mathrm{PPO}=$ Peak power output.

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participation and an increase in the aerobic system participation. Recently, Panissa et al. (2018) studied a short-form of HIIE, and their findings also reinforced the raising of aerobic contribution during HIIE. Most of the findings regarding energy systems contributions could be related to training volume, total effort duration, or, specifically in HIIE, the sum of repeated efforts (Buchheit \& Laursen, 2013b). As these variables increase, the contribution of the aerobic system also rises (Gastin, 2001; Glaister, 2005; Trump et al., 1996).

Based on this, it was not surprising that the protocols showed high aerobic contribution (Gaitanos et al., 1993). Even the low exercise duration, found in the $170 \% \mathrm{PPO}$ condition, had an average length of approximately 120 s . About $1 / 3$ of this time involved pause periods, in which aerobic contribution is very high (Brooks \& Mercier, 1994). The more substantial aerobic contribution found in the $115 \% \mathrm{PPO}$ would be explained by the relatively lower required anaerobic contribution for lower exercise intensities. Furthermore, $115 \% \mathrm{PPO}$ allowed more repetitions, contributing to a longer duration in the session ( 488 s , vs 258 and 128 in $130 \%$ PPO and $170 \% \mathrm{PPO}$, respectively). We also observed that, as the intensity increases, the anaerobic systems exert a higher relative, but not absolute, contribution. This higher relative contribution might have occurred because, when exercising at higher intensities, it allowed a lower number of bouts, reducing the total duration of the effort.

Despite the high blood [La-], frequently higher than $11 \mathrm{mmol} . \mathrm{L}^{-1}$, the relative glycolytic contribution was not so high, probably since each lactate unit corresponds to only $3 \mathrm{ml} . \mathrm{O}_{2}$. $\mathrm{kg}^{-1}$ (Bertuzzi et al., 2016; Bertuzzi et al., 2007; Campos et al., 2012), and the participants had higher oxygen consumption than accumulated lactate, even after respective conversion. This fact reinforces the hypothesis that measuring the glycolytic contribution of a given
exercise considering only the blood [La-] might not be recommended (Bertuzzi et al., 2016), or the glycolytic contribution is quite small during some HIIE (Gaitanos et al., 1993; Trump et al., 1996). Another possibility is that a greater amount of lactate was metabolised within the muscle when the exercise was longer (Brooks, 1986); possibly reducing the lactic contribution. It is important to highlight that the assumption of equating $1 \mathrm{mmol} . \mathrm{L}^{-1}$ of lactate to $3 \mathrm{ml} . \mathrm{O}_{2} . \mathrm{kg}^{-1}$ stands for submaximal exercises. However, many other studies used that to assess supramaximal exercises lactic contribution (Bertuzzi et al., 2007; Campos et al., 2012; Lopes-Silva et al., 2015). Further comparisons using direct measurements (i.e., muscle biopsy) should be made to confirm the reliability and replicability of this method.

The athletes presented higher blood $\left[\mathrm{La}^{-}\right]$for all intensities, therefore, also presented a higher absolute lactic contribution, this occurred probably due to the greater anaerobic capacity of this population (Ponorac et al., 2007), which contributed to the higher power output during the efforts. Regarding oxygen consumption in athletes, the highest value was in $130 \%$ PPO. Considering that maintaining higher $\mathrm{V} \cdot \mathrm{O}_{2}$ values during exercise is essential for $\mathrm{V} \cdot \mathrm{O}_{2 \mathrm{MAX}}$ increase (Midgley \& Mc Naughton, 2006), the optimal intensity for aerobic power development in athletes, in this time structure, would be near to $130 \%$ PPO. Previously, on a treadmill, other studies demonstrated that for $30 \mathrm{~s}: 15 \mathrm{~s}$ training in active young men and $15 \mathrm{~s}: 15 \mathrm{~s}$ in middle-aged runners, the intensities of $110 \% \mathrm{vV} \cdot \mathrm{O}_{2 \mathrm{MAX}}$ and $100 \% \mathrm{vV} \cdot \mathrm{O}_{2 \mathrm{MAX}}$, respectively, presented the higher oxygen consumption (Aguiar et al., 2013; Billat et al., 2001). Otherwise, subjects maintained the $\mathrm{V} \cdot \mathrm{O}_{2}$ closed to $90-95 \%$ of the $\mathrm{V} \cdot \mathrm{O}_{2 \mathrm{MAX}}$, which is lower than the $100 \%$ reached in the original study and questioned the author's statement that the 'Tabata training' should be considered as "one of the most energetically effective exercise training protocols for maximally improving both the aerobic and anaerobic energy-supplying systems" (Tabata, 2019).

Interestingly, at the initially suggested intensity of $170 \% \mathrm{~V} \cdot \mathrm{O}_{2 \mathrm{Max}}$ (Tabata et al., 1996), most of the athletes and non-athletes were unable to complete the $7-8$ sprints. This aspect was previously questioned (Viana et al., 2018c), and these incompatibilities are possibly due to the differences between the tests used to obtain the training loads (Tabata, 2019), which put some light in this debatable point from the findings of the present investigation. While Tabata et al. (1996) used an obsolete, unpractical and questionable protocol that requires a large number of $10-\mathrm{min}$ visits to the laboratory (Bangsbo, 1992; Medbo et al., 1988), Viana et al. (2018c) we used the traditional, worldwide-used, and very practical maximal graded exercise test (Buchheit \& Laursen, 2013b).

Even using a similar graded testing protocol, our findings are different from those found by Viana et al. (2018c), in which the intensity of $115 \%$ PPO allowed the accomplishment of $7 \pm 1$ sprints, our results suggest that it is possible to perform this same number of bouts at a higher intensity ( $130 \% \mathrm{PPO}$ ). Probably this difference is due to the type of ergometer used since previous authors used a magnetic locking model. In contrast, the present study used a mechanical braking device, similar to that used by Tabata et al. (1996). Such differences further reinforce the problem of generalisation of interval protocols reported in previous studies (Viana et al., 2018c), especially regarding the protocol proposed by Tabata et al. (Tabata et al., 1996; Viana et al., 2018a).

There seems to be an apparent conflict in the structural variables of the HIIE protocol with the 20s:10s effort:pause structure as commonly used (Tabata et al., 1996). According to previous studies, we classify as a short HIIE model (effort and pause block lasting less than one minute). However, the intensity close to $170 \% \mathrm{~V} \cdot \mathrm{O}_{2 \text { MAX }}$ is characteristic of Sprint Interval Training models (Buchheit \& Laursen, 2013a). Notwithstanding, the effort:pause ratio for sprint interval training is 1:4-8 due to the need to recover anaerobic pathways to maintain an elevated power output (Brooks \& Mercier, 1994; Glaister, 2005), whereas in the classic $20 \mathrm{~s}: 10 \mathrm{~s}$ protocol this ratio is $2: 1$. This conflict might explain why it was not possible to reach $7-8$ bouts when using $20 \mathrm{~s}: 10$ s, with a load equivalent to $170 \% \mathrm{~V} \cdot \mathrm{O}_{2 \mathrm{MAX}}$, even in athletes.

Finally, despite the methodological issues that we should consider regarding this specific HIIE protocol (Gentil et al., 2016; Tabata et al., 1996; Viana et al., 2018c), its potential to induce positive physiological changes should be recognised (Ma et al., 2013; MiyamotoMikami et al., 2018; Scribbans et al., 2014; Tabata et al., 1996). Both "classical" studies by the "Tabata" group showed that participants could achieve relevant aerobic and anaerobic improvements in a very high time-efficient manner (Tabata et al., 1996). More recently, the same group showed that this protocol could significantly increase aerobic power, maximal accumulated oxygen deficit, and thigh muscle cross-sectional area (Miyamoto-Mikami et al., 2018). Further longitudinal studies should investigate this 20 s:10s protocol at an intensity range of 115 to $130 \% \mathrm{PPO}$ in order to raise the time near and at $\mathrm{V} \cdot \mathrm{O}_{2 \text { max }}$.

## CONCLUSIONS

In conclusion, to a $20 \mathrm{~s}: 10 \mathrm{~s}$ HIIE protocol, the aerobic contribution is predominant, independently of the intensity applied in a range from $115 \%$ PPO to $170 \%$ PPO. Despite that, the lower the intensity, the higher is the aerobic contribution, and $130 \% \mathrm{PPO}$ is the suggested intensity to induce higher V.O2 in trained cyclists. Finally, to reach about eight sprints, we propose the intensity of $130 \%$ the power at $\mathrm{V} \cdot \mathrm{O}_{2 \text { MAX }}$ obtained in a graded test, either for athletes or non-athletes.

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## Competing Interests

The authors declare there are no competing interests.

## Author Contributions

- Gabriel V. Protzen conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Charles Bartel, Victor S. Coswig and Paulo Gentil conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Fabricio B. Del Vecchio conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.


## Human Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

The Federal University of Pelotas Ethical Committee approved this research project (77729517.1.0000.5313).

## Data Availability

The following information was supplied regarding data availability:
All data are available in the Supplementary Files.

## Supplemental Information

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## REFERENCES

Aguiar R, Schlickmann J, Turnes T, Caputo F. 2013. Efeito da intensidade do exercício de corrida intermitente $30 \mathrm{~s}: 15 \mathrm{~s}$ no tempo de manutenção no ou próximo do VO2max. Motriz 19:207-216 DOI 10.1590/S1980-65742013000100021.
Bangsbo J. 1992. Is the O2 deficit an accurate quantitative measure of the anaerobic energy production during intense exercise? Journal of Applied Physiology 73:1207-1209 DOI 10.1152/jappl.1992.73.3.1207.
Bertuzzi RC, Franchini E, Kokubun E, Kiss MA. 2007. Energy system contributions in indoor rock climbing. European Journal of Applied Physiology and Occupational Physiology 101:293-300 DOI 10.1007/s00421-007-0501-0.
Bertuzzi R, Melegati J, Bueno S, Ghiarone T, Pasqua LA, Gaspari AF, Lima-Silva AE, Goldman A. 2016. GEDAE-LaB: a free software to calculate the energy system contributions during exercise. PLOS ONE 11:e0145733 DOI 10.1371/journal.pone.0145733.
Billat VL, Slawinksi J, Bocquet V, Chassaing P, Demarle A, Koralsztein JP. 2001. Very short ( $15 \mathrm{~s}-15 \mathrm{~s}$ ) interval-training around the critical velocity allows middle-aged runners to maintain VO2 max for 14 min . International Journal of Sports Medicine 22:201-208 DOI 10.1055/s-2001-16389.

Bonafiglia JT, Edgett BA, Baechler BL, Nelms MW, Simpson CA, Quadrilatero J, Gurd BJ. 2017. Acute upregulation of PGC-1alpha mRNA correlates with training-induced increases in SDH activity in human skeletal muscle. Applied Physiology, Nutrition, and Metabolism 42:656-666 DOI 10.1139/apnm-2016-0463.
Brooks GA. 1986. The lactate shuttle during exercise and recovery. Medicine and Science in Sports and Exercise 18:360-368 DOI 10.1249/00005768-198606000-00019.
Brooks GA, Mercier J. 1994. Balance of carbohydrate and lipid utilization during exercise: the crossover concept. Journal of Applied Physiology 76:2253-2261 DOI 10.1152/jappl.1994.76.6.2253.
Buchheit M, Laursen PB. 2013a. High-intensity interval training, solutions to the programming puzzle. Part II: anaerobic energy, neuromuscular load and practical applications. Sports Medicine 43:927-954 DOI 10.1007/s40279-013-0066-5.
Buchheit M, Laursen PB. 2013b. High-intensity interval training, solutions to the programming puzzle: Part I: cardiopulmonary emphasis. Sports Medicine 43:313-338 DOI 10.1007/s40279-013-0029-x.
Campos FA, Bertuzzi R, Dourado AC, Santos VG, Franchini E. 2012. Energy demands in taekwondo athletes during combat simulation. European Journal of Applied Physiology and Occupational Physiology 112:1221-1228 DOI 10.1007/s00421-011-2071-4.
Di Prampero PE, Ferretti G. 1999. The energetics of anaerobic muscle metabolism: a reappraisal of older and recent concepts. Respiration Physiology 118:103-115 DOI 10.1016/S0034-5687(99)00083-3.
Domaradzki J, Cichy I, Rokita A, Popowczak M. 2020. Effects of tabata training during physical education classes on body composition, aerobic capacity, and anaerobic performance of under-, normal- and overweight adolescents. International Journal of Environmental Research and Public Health 17:876-887 DOI 10.3390/ijerph17030876.
Gaitanos GC, Williams C, Boobis LH, Brooks S. 1993. Human muscle metabolism during intermittent maximal exercise. Journal of Applied Physiology 75:712-719 DOI 10.1152/jappl.1993.75.2.712.
Gastin PB. 2001. Energy system interaction and relative contribution during maximal exercise. Sports Medicine 31:725-741 DOI 10.2165/00007256-200131100-00003.
Gentil P, Naves JP, Viana RB, Coswig V, Dos Santos Vaz M, Bartel C, Del Vecchio FB. 2016. Revisiting Tabata's protocol: does it even exist? Medicine and Science in Sports and Exercise 48:2070-2071 DOI 10.1249/MSS.0000000000001023.
Giles D, Draper N, Neil W. 2016. Validity of the Polar V800 heart rate monitor to measure RR intervals at rest. European Journal of Applied Physiology and Occupational Physiology 116:563-571 DOI 10.1007/s00421-015-3303-9.
Glaister M. 2005. Multiple sprint work: physiological responses, mechanisms of fatigue and the influence of aerobic fitness. Sports Medicine 35:757-777 DOI 10.2165/00007256-200535090-00003.
Haseler LJ, Hogan MC, Richardson RS. 1999. Skeletal muscle phosphocreatine recovery in exercise-trained humans is dependent on O2 availability. Journal of Applied Physiology 86:2013-2018 DOI 10.1152/jappl.1999.86.6.2013.

Logan GR, Harris N, Duncan S, Plank LD, Merien F, Schofield G. 2016. Low-active male adolescents: a dose response to high-intensity interval training. Medicine and Science in Sports and Exercise 48:481-490 DOI 10.1249/MSS.0000000000000799.
Lopes-Silva JP, Silva Santos JFD, Branco BHM, Abad CCC, Oliveira LFD, Loturco I, Franchini E. 2015. Caffeine ingestion increases estimated glycolytic metabolism during taekwondo combat simulation but does not improve performance or parasympathetic reactivation. PLOS ONE 10:e0142078 DOI 10.1371/journal.pone.0142078.
Ma JK, Scribbans TD, Edgett BA, Boyd JC, Simpson CA, Little JP, Gurd BJJOJOM, Physiology I. 2013. Extremely low-volume, high-intensity interval training improves exercise capacity and increases mitochondrial protein content in human skeletal muscle. Open Journal of Molecular and Integrative Physiology 3:202-210 DOI 10.4236/ojmip.2013.34027.
MacInnis MJ, Gibala MJ. 2017. Physiological adaptations to interval training and the role of exercise intensity. Journal de Physiologie 595:2915-2930 DOI 10.1113/JP273196.
Margaria R, Cerretelli P, Diprampero PE, Massari C, Torelli G. 1963. Kinetics and mechanism of oxygen debt contraction in man. Journal of Applied Physiology 18:371-377 DOI 10.1152/jappl.1963.18.2.371.
McRae G, Payne A, Zelt JG, Scribbans TD, Jung ME, Little JP, Gurd BJ. 2012. Extremely low volume, whole-body aerobic-resistance training improves aerobic fitness and muscular endurance in females. Applied Physiology, Nutrition, and Metabolism 37:1124-1131 DOI 10.1139/h2012-093.
Medbo JI, Mohn AC, Tabata I, Bahr R, Vaage O, Sejersted OM. 1988. Anaerobic capacity determined by maximal accumulated O2 deficit. Journal of Applied Physiology 64:50-60 DOI 10.1152/jappl.1988.64.1.50.
Midgley AW, Mc Naughton LR. 2006. Time at or near VO2max during continuous and intermittent running. A review with special reference to considerations for the optimisation of training protocols to elicit the longest time at or near VO2max. Journal of Sports Medicine and Physical Fitness 46:1-14.
Miyamoto-Mikami E, Tsuji K, Horii N, Hasegawa N, Fujie S, Homma T, Uchida M, Hamaoka T, Kanehisa H, Tabata I, Iemitsu M. 2018. Gene expression profile of muscle adaptation to high-intensity intermittent exercise training in young men. Scientific Reports 8:16811 DOI 10.1038/s41598-018-35115-x.
Panissa VLG, Fukuda DH, Caldeira RS, Gerosa-Neto J, Lira FS, Zagatto AM, Franchini E. 2018. Is oxygen uptake measurement enough to estimate energy expenditure during high-intensity intermittent exercise? Quantification of anaerobic contribution by different methods. Frontiers in Physiology 9:868 DOI 10.3389/fphys.2018.00868.
Ponorac N, Matavulj A, Rajkovaca Z, Kovacevic P. 2007. [The assessment of anaerobic capacity in athletes of various sports]. Medicinski Pregled 60:427-430 DOI 10.2298/MPNS0710427P.
Schaun GZ, Del Vecchio FB. 2018. High-intensity interval exercises' acute impact on heart rate variability: comparison between whole-body and cycle ergometer protocols. The Journal of Strength and Conditioning Research 32:223-229 DOI 10.1519/JSC.0000000000002180.

Scribbans TD, Edgett BA, Vorobej K, Mitchell AS, Joanisse SD, Matusiak JB, Parise G, Quadrilatero J, Gurd BJ. 2014. Fibre-specific responses to endurance and low volume high intensity interval training: striking similarities in acute and chronic adaptation. PLOS ONE 9:e98119 DOI 10.1371/journal.pone.0098119.
Tabata I. 2019. Tabata training: one of the most energetically effective high-intensity intermittent training methods. Journal of Physiological Sciences 69:559-572 DOI 10.1007/s12576-019-00676-7.
Tabata I, Nishimura K, Kouzaki M, Hirai Y, Ogita F, Miyachi M, Yamamoto K. 1996. Effects of moderate-intensity endurance and high-intensity intermittent training on anaerobic capacity and VO2max. Medicine and Science in Sports and Exercise 28:1327-1330 DOI 10.1097/00005768-199610000-00018.
Trump ME, Heigenhauser GJ, Putman CT, Spriet LL. 1996. Importance of muscle phosphocreatine during intermittent maximal cycling. Journal of Applied Physiology 80:1574-1580 DOI 10.1152/jappl.1996.80.5.1574.
Viana RB, De Lira CAB, Naves JPA, Coswig VS, Del Vecchio FB, Gentil P. 2018a. Tabata protocol: a review of its application, variations and outcomes. Clinical Physiology and Functional Imaging 39:1-8 DOI 10.1111/cpf. 12513.
Viana RB, De Lira CAB, Naves JPA, Coswig VS, Del Vecchio FB, Ramirez-Campillo R, Vieira CA, Gentil P. 2018b. Can we draw general conclusions from interval training studies? Sports Medicine 48:2001-2009 DOI 10.1007/s40279-018-0925-1.
Viana RB, Naves JP, De Lira CA, Coswig VS, Del Vecchio FB, Vieira CA, Gentil P. 2018c. Defining the number of bouts and oxygen uptake during the Tabata protocol performed at different intensities. Physiology and Behavior 189:10-15 DOI 10.1016/j.physbeh.2018.02.045.
Zagatto AM, Redkva PE, De Poli RAB, Gonzalez JAM, Brandani JZ, Penedo T, Bertuzzi RCM. 2019. 3-min all-out effort on cycle ergometer is valid to estimate the anaerobic capacity by measurement of blood lactate and excess postexercise oxygen consumption. European Journal of Sport Science 19:645-652 DOI 10.1080/17461391.2018.1546338.

