**Daniel Appelhans** 

# Investigating individual differences in the acute physiological response during high-intensity interval training in well-trained athletes

Master's thesis in Physical Activity and Health - Specialization in Exercise Physiology Supervisor: Knut Skovereng May 2021

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# Investigating individual differences in time above 90%VO2<sub>max</sub> during HIIT



Large Individual differences in T > 90%VO2<sub>max</sub> observed in previous studies might be due to fixed intensity prescription instead of physiology. IM intervals should be time-matched as sets, not only using the "hard efforts".

Implications

Using constant best average will elicit higher physiological response and equalize T > 90%VO2<sub>max</sub> between IM and 6x5min.

## Abstract

In previous studies comparing different interval protocols, large individual differences in time above 90% of maximal oxygen consumption (t > 90% $\dot{V}O2_{max}$ ) have been observed. Even though t > 90% $\dot{V}O2_{max}$  is regarded as an important parameter in quantifying the effectiveness of high-intensity interval training (HIIT), the reason for this variation has not been investigated. Therefore, this master thesis aims at investigating the individual differences in. t > 90% $\dot{V}O2_{max}$ in well-trained athletes between different interval protocols and modes of intensity prescription

Twelve well-trained cyclists and triathletes ( $\dot{V}O2_{max}$ : 68 ± 6.3 L·min<sup>-1</sup>·kg<sup>-1</sup>) performed two different interval protocols of 3x13x30/15-seconds (IM) and 6x5-minutes (TRAD) twice in a randomized order. Each protocol was first performed with a fixed intensity (Fixed) based on percentages of maximal aerobic power (MAP) and the next time self-paced with a maximal session effort (Free).

Neither fractional utilization of  $\dot{VO2}_{max}$  ( $\dot{VO2}_{max}@AT$ ) nor time-to-exhaustion at MAP (T<sub>lim</sub>) was correlated with the t > 90 $\dot{VO2}_{max}$  in any of the sessions. Additionally, no other physiological parameter assessed in this study could predict t > 90 $\dot{VO2}_{max}$ . The coefficient of variation (CV) for t > 90 $\dot{VO2}_{max}$  was lower for IM (18%) and TRAD (31%) in Free compared to Fixed (IM: 62% and TRAD: 90%), and all physiological parameters assessed were higher in Free than Fixed (all p < 0.001; all  $\eta_p^2$  > 0.795). Every participant could work at a higher intensity in Free, but the degree of work intensity was individual. In IM, this degree of a greater work rate was positively correlated to the change in t > 90 $\dot{VO2}_{max}$  between the two exercise modes (p < 0.05; r = 0.669). The t > 90 $\dot{VO2}_{max}$  was significantly higher for IM than TRAD in Fixed (p < 0.01) but not in Free (p = 0.321).

The large individual differences in t >  $90\%\dot{V}O2_{max}$  following a HIIT session seem rather influenced by the methodology of prescribing a fixed intensity instead of differences in physiological parameters. Using the self-paced maximal session effort and a time-matching which includes the 15-seconds rest of IM as interval time, reduces the physiological differences between IM and TRAD, indicating a more uniform cardiovascular stimulus than previously reported.

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# Table of Contents

|   | List o         | of Figuresxi                                  | ii |  |  |
|---|----------------|---|----|--|--|
|   | List of Tables |   |    |  |  |
|   | List o         | of Abbreviationsx                             | v  |  |  |
| 1 | Inti           | troduction                                    | 1  |  |  |
|   | 1.1            | Methodological Considerations                 | 2  |  |  |
|   | 1.2            | Physiological Considerations                  | 3  |  |  |
|   | 1.3            | Session Considerations                        | 3  |  |  |
|   | 1.4            | Aim   | 4  |  |  |
| 2 | Me             | ethods  | 5  |  |  |
|   | 2.1            | Subjects                                      | 5  |  |  |
|   | 2.2            | Study Outline                                 | 6  |  |  |
|   | 2.3            | Testing Procedures                            | 6  |  |  |
|   | 2.3            | 3.1 Instrumentation                           | 7  |  |  |
|   | 2.3            | 3.2 Experimental protocol                     | 7  |  |  |
|   | 2              | 2.3.2.1 Test battery                          | 7  |  |  |
|   | 2              | 2.3.2.2 Interval Sessions                     | Э  |  |  |
|   | 2.4            | Data analysis1                                | 1  |  |  |
|   | 2.5            | Statistical Analysis1                         | 2  |  |  |
| 3 | Res            | sults14                                       | 4  |  |  |
|   | 3.1            | Comparison between Interval Sessions14        | 4  |  |  |
|   | 3.2            | Correlation Analysis                          | 1  |  |  |
| 4 | Dis            | scussion                                      | 3  |  |  |
|   | 4.1            | Physiological Factors                         | 3  |  |  |
|   | 4.2            | Methodological Factors24                      | 4  |  |  |
|   | 4.3            | Comparison IM and TRAD 20                     | 6  |  |  |
|   | 4.4            | Strength, Limitations, and Future Perspective | Э  |  |  |
|   | 4.5            | Conclusion                                    | C  |  |  |
| R | eferer         | nces  | 2  |  |  |
| A | ppend          | dices   | 5  |  |  |

# List of Figures

| Figure 1. Study Outline  | . 6 |
|--|-----|
| Figure 2. Test Day One   | . 8 |
| Figure 3. Test Day Two   | . 9 |
| Figure 4. Outline interval sessions.   | 11  |
| Figure 5. Oxygen uptake (VO2) during the different interval sessions                             | 15  |
| Figure 6. Power output during the different interval sessions.                                   | 15  |
| Figure 7. Time spent at different percentages of VO2 <sub>max</sub> during the interval sessions | 16  |
| Figure 8. Individual and mean time above 90% VO2 <sub>max</sub>                                  | 17  |
| Figure 9. Individual and mean time above 90% HR <sub>max</sub>                                   | 18  |
| Figure 10. Physiological response parameters at the different interval time points               | 20  |
| Figure 11. Relationship between %VO2 <sub>max</sub> @AT and t > 90% VO2 <sub>max</sub>           | 21  |
| Figure 12. Relationship between T <sub>lim</sub> and t > 90% VO2 <sub>max</sub>                  | 22  |
| Figure 13. Relationship between the change of t > 90% VO2 <sub>max</sub> and MAP                 | 22  |

# List of Tables

| Table 1. Subject characteristics  | 5  |
|---|----|
| Table 2. Training and racing background   | 5  |
| Table 3. Preliminary testing results  | 14 |
| Table 4. Two-Way ANOVA and post-hoc comparisons for all parameters in IM and TRAD | 19 |

# List of Abbreviations

| AT                        | Anaerobic threshold  |
|---------------------------|--|
| AT-effort                 | 15-minute anaerobic threshold effort                               |
| CV                        | Coefficient of variation   |
| DEC                       | Decreased intervals  |
| Fixed                     | Fixed intensities (based on MAP)                                   |
| Free                      | Self-paced intensity based on the maximal session effort           |
| f <sub>R</sub>            | Breathing frequency  |
| HIIT                      | High-intensity interval training                                   |
| HIT                       | High-intensity training  |
| HR                        | Heart rate   |
| IM                        | Intermittent interval (sessions)/ 13x30/15-seconds                 |
| iVO2 <sub>max</sub>       | maximal aerobic power/speed/velocity at VO2 <sub>max</sub>         |
| Lac <sub>min</sub>        | Lactate minimum (derived from LMT)                                 |
| LMT                       | Lactate minimum test   |
| LT                        | Lactate profile test   |
| NSD                       | Norwegian social science data services                             |
| РРО                       | Peak power output  |
| RPE                       | Rating of perceived exhaustion                                     |
| RER                       | Respiratory exchange ratio   |
| sRPE                      | Session RPE  |
| T <sub>lim</sub> (at MAP) | Time-to-exhaustion (at maximal aerobic power)                      |
| TRAD                      | Traditional interval (sessions) / 6x5-minutes                      |
| t > 90%HR <sub>max</sub>  | Time above 90% maximal heart rate                                  |
| t > 90%VO2 <sub>max</sub> | Time above 90% maximal oxygen consumption                          |
| VCO2                      | Carbon dioxide production  |
| ν̈́Ε                      | Minute Ventilation   |
| VO2                       | Oxygen consumption   |
| VO2 <sub>max</sub>        | Maximal oxygen consumption   |
| %VO2 <sub>max</sub>       | Fractional utilization of VO2 <sub>max</sub>                       |
| %VO2 <sub>max</sub> @ AT  | Fractional utilization of $\dot{V}O2_{max}$ at anaerobic threshold |

## 1 Introduction

Exercise and training are an integral part of successful sports performance. In endurance sports, world-class athletes train up to 20-hours per week,<sup>1</sup> and top-level cyclists cover between 30.000 – 35.000km per year.<sup>2</sup> Around 80% of this total training volume is classified as low-intensity training, and 20% is performed at higher intensities, exceeding lactate values of 2mmol·L<sup>-1</sup>.<sup>3</sup> Even though both intensity domains are recognized as essential in developing endurance performance,<sup>4</sup> for well-trained athletes the 20% can be further specified into threshold and high-intensity training (HIT).<sup>3</sup> Training interventions comparing these two intensities showed beneficial effects on endurance performance, especially following HIT.<sup>5</sup>

From a physiological standpoint, endurance performance depends on the three key variables maximal oxygen consumption ( $\dot{V}O2_{max}$ ), fractional utilization of  $\dot{V}O2_{max}$  ( $\%\dot{V}O2_{max}$ ), and work economy/efficiency.<sup>6,7</sup> The  $\%\dot{V}O2_{max}$  is thereby defined as the highest percentage of  $\dot{V}O2_{max}$  that can be sustained for a given exercise duration, which for longer events, is closely related to the  $\%\dot{V}O2_{max}$  at the anaerobic threshold (AT).<sup>6</sup> While the AT's exact determination and terminology as a state of maximal physiological homeostasis is debated,<sup>8–10</sup> the importance of the concept for endurance performance is generally acknowledged.<sup>8</sup> The  $\dot{V}O2_{max}$  is, in comparison, less disputed, and its significant role in endurance performance was first identified as early as 1920.<sup>6</sup> It is "the highest rate at which oxygen can be taken up and utilized by the body under severe exercise"<sup>6,p.70</sup> and defined by the Fick equation: the product of the stroke volume (SV) and heart rate (HR), termed as cardiac output (CO), divided by the arteriovenous oxygen difference. The improvement in  $\dot{V}O2_{max}$  following exercise training is mainly attributed to an increased SV (and thus CO).<sup>6,11,12</sup>

As a critical factor in determining performance in endurance events, many studies investigated the effects of different training regimes on the  $\dot{V}O2_{max}$ .<sup>13</sup> High-intensity interval training (HIIT) was identified as a suitable method to increase  $\dot{V}O2_{max}$  across all ability levels from patients, old, sedentary, trained, well-trained, and elite subjects.<sup>14–16</sup> Thereby, different forms of HIIT have been developed, including short aerobic intervals (< 1-minute) and traditionally longer aerobic intervals (1 to 8-minutes), separated by shorter rest periods of approximately half the work duration.<sup>17,18</sup> Recently, slightly different designs, including intermittent<sup>19</sup> and varied intervals<sup>20</sup> were created to potentially increase VO2max even more effectively.

Within HIIT, an intensity close to  $\dot{VO2}_{max}$  has been deemed necessary to elicit significant improvements.<sup>21</sup> As evidence suggests that SV rises to  $\dot{VO2}_{max}^{22}$  and mechanical overload is the primary stimulus for morphological adaptation of the myocardium,<sup>23</sup> the accumulated time near  $\dot{VO2}_{max}$  seems essential for the positive adaptations following HIIT.<sup>13,24</sup> The correlation between  $\dot{VO2}$  and HR suggests that equal importance can be attributed to the HR response.<sup>25</sup> Therefore, the time above  $90\%\dot{VO2}_{max}$  (t >  $90\%\dot{VO2}_{max}$ )<sup>21,26</sup> and the time above 90% maximal heart rate (t >  $90\%HR_{max}$ )<sup>27</sup> are used to quantify the quality of interval sessions. However, studies investigating the acute effect of different HIIT sessions designed to elicit the highest t >  $90\%\dot{VO2}_{max}$  showed substantial individual differences in the accumulated time.<sup>19,20,28,29</sup> Bossi et al.<sup>20</sup> have shown differences in t >  $90\%\dot{VO2}_{max}$ , ranging from under one minute to more than 10 minutes, and Almquist et al.<sup>29</sup> from four to 25-minutes. The factors influencing these large individual differences are not clear yet<sup>28</sup> but could be of methodological or physiological origin.

#### 1.1 Methodological Considerations

The minimum speed/ power to attain  $\dot{VO}2_{max}$  (maximal aerobic power/speed/velocity at  $\dot{VO}2_{max} = i\dot{VO}2_{max}$ ) or percentages of that value are usually used to prescribed intensity in HIIT sessions<sup>17,18</sup> but using this kind of intensity prescription might not be accurate across individuals.<sup>30,31</sup> Scharhag-Rosenberger et al.<sup>32</sup> showed that training prescriptions based on percentages of  $\dot{VO}2_{max}$  resulted in different metabolic strain due to individual lactate accumulation, which could affect exercise tolerance and subsequently the individual t > 90% $\dot{VO}2_{max}$ . In recent exercise interventions, the "maximal overall session effort" (isoeffort) or best-effort approach was applied for prescribing interval intensity to resemble the training approach athletes typically use and possibly account for individual variations.<sup>27,33</sup> The isoeffort approach lets the athlete self-pace by choosing the right intensity to complete the desired workout based on their perceived exhaustion and experience.<sup>34</sup> However, this approach might only be suited if a valid intensity target is provided or when the athletes are already well-trained and familiar with HIIT due to the reliance on previous experience.

## 1.2 Physiological Considerations

Rønnestad et al.<sup>28</sup> suggest that individual differences in the fractional utilization of  $\dot{VO2}_{max}$  at the anaerobic threshold ( $\dot{VO2}_{max}$ @AT) could account for the large inter-individual variation observed in t > 90 $\dot{VO2}_{max}$ , and Coyle et al.<sup>35</sup> showed that the time-to-exhaustion at 88% of  $\dot{VO2}_{max}$  was longer for well-trained cyclists with a high  $\dot{VO2}_{max}$ @AT. For HIIT, a higher  $\dot{VO2}_{max}$ @AT (e.g., close to 90%) might allow athletes to spend more t > 90 $\dot{VO2}_{max}$  because the necessary intensity is not much higher than their AT.

Another factor that could account for the differences is time-to-exhaustion ( $T_{lim}$ ) at maximal aerobic intensity. The critical power model from Monod and Scherer illustrates that a greater difference between AT and maximal aerobic power (MAP) results in a reduced duration of  $T_{lim}$ at MAP.<sup>36</sup> When performing HIIT, a greater difference between AT and i $\dot{V}O2_{max}$  may attenuate the build-up of metabolites and thus potentially reducing t > 90% $\dot{V}O2_{max}$ .

### 1.3 Session Considerations

Especially in cycling practice, intermittent intervals (IM) with short but intense work periods and only a 2:1 recovery ratio within a work set of multiple repetitions are widely used. Due to the multiple short rest periods within a work set, higher power can be maintained over a longer time than in traditional intervals without cardiovascular parameters like the HR and oxygen consumption ( $\dot{V}O2$ ) dropping significantly.<sup>19</sup> Previous studies found that physiological responses like t > 90% $\dot{V}O2_{max}$  and t > 90%HR<sub>max</sub> are significantly higher in an IM session (3x13x30/15-seconds) compared to a more traditional 4x5-minutes interval session.<sup>29</sup> Almquist et al.<sup>29</sup> matched these intervals time and effort-wise, but the time matching was done only using the 30-seconds during the intermittent intervals, equaling 19.5-minutes. In cycling practice, however, IM intervals are usually viewed in terms of the whole set of 13x30/15-seconds because only a little regeneration on a muscular but not cardiovascular level, due to the alternating nature of high work and short rest periods, is wanted. If therefore, the whole set (9.75-minutes) is treated as one interval instead of only the 30-seconds, a session of 3x13x30/15-seconds should be compared to ca. 30-minutes of interval time in a traditional session design.

## 1.4 Aim

In the past, research in exercise science has focused on identifying the most suitable HIIT session in general, but the reason for large individual differences in the physiological important HIIT parameter t > 90% $\dot{V}O2_{max}$  across multiple studies has not been identified yet. Therefore, this master thesis aims at investigating the individual differences in time accumulated above 90%  $\dot{V}O2_{max}$  in well-trained athletes between different interval protocols and modes of intensity prescription. As a secondary aim, the physiological response to an intermittent interval protocol of 3x13x30/15-seconds is compared to a traditional 6x5-minutes protocol. The hypothesis is that intensity prescription based on the fixed values of MAP will cause greater individual differences in t > 90% $\dot{V}O2_{max}$ . However, differences in the physiological parameters  $\%\dot{V}O2_{max}@AT$  and  $T_{lim}$  will also explain time differences in the best effort approach.

## 2 Methods

## 2.1 Subjects

Fourteen well-trained male cyclists and triathletes participated in this study. The final data analysis was completed with twelve participants. Two data sets were excluded due to measurement errors with one participant and another not completing all sessions. Subject characteristics are presented in Table 1. According to the criteria recommended by De Pauw et al.<sup>37</sup>, the participants were categorized as well-trained endurance athletes ("performance level 4"). All participants were experienced with interval and bike training (Table 2). The study was approved by the Norwegian Social Science Data Services (NSD) and conducted according to the ethical standards by the Helsinki Declaration of 1976. Before providing written consent, the participants were informed about possible risks and their right to withdraw from the study at any point.

| Age (years)  | 23.7 ± 1.9                       | [21 – 28]      |
|--|----------------------------------|----------------|
| Height (cm)  | $178\pm4.9$                      | [170 – 189]    |
| Body mass (kg)                                       | $\textbf{73.1} \pm \textbf{9.6}$ | [58.4 – 91.7]  |
| VO2 <sub>max</sub> (L∙min⁻¹)                         | $4.9\pm0.5$                      | [3.9 – 5.5]    |
| VO2 <sub>max</sub> (L∙min⁻¹·kg⁻¹)                    | $68\pm 6.3$                      | [59.6 – 81.32] |
| Absolute Peak Power Output PPO (W)                   | $425\pm56$                       | [323 – 500]    |
| Relative Peak Power Output PPO (W·kg <sup>-1</sup> ) | $5.8\pm0.6$                      | [4.9 – 7.1]    |

| Table 1. Su | bject chara | acteristics |
|-------------|-------------|-------------|
|-------------|-------------|-------------|

Values are presented as mean  $\pm$  standard deviation and [minimum – maximum].

### Table 2. Training and racing background

| Training time (hours per week)              | $11.2\pm2.8$ | [6.5 – 16.5] |
|---|--------------|--------------|
| Interval sessions (per week)                | $2\pm0.8$    | [0.5 – 3]    |
| Experience interval training (Scale 1 - 10) | $7.7\pm1.8$  | [4 - 10]     |
| Bike races (last season)                    | $2.5\pm2.9$  | [0-8]        |
| Experience bike racing (Scale 1 - 10)       | $6.2\pm2.8$  | [2 – 10]     |

Values are presented as mean  $\pm$  standard deviation and [minimum – maximum]. All values are self-reported answers and were obtained through an own designed questionnaire (attached in the appendix).

## 2.2 Study Outline

The study included a total of six test sessions per participant, and each session was separated by at least 48-hours to ensure adequate recovery. A physiological test battery was performed on the first two days, and days three to six were allocated to the different interval sessions (Figure 1). These sessions included two different protocols, which were performed twice – one time with a fixed intensity (Fixed) and the other with isoeffort/maximal session effort intensity (Free). The order of the two protocols was randomized, but the Fixed session always preceded the Free one (Figure 1).



**Figure 1. Study Outline.** A total of six testing days was performed per participant. A lactate profile test (LT), a  $\dot{VO2}_{max}$  ramp test and a lactate minimum test (LMT) on the first day, and a 15-minute anaerobic threshold effort (AT-Effort) and time-to-exhaustion test at MAP (T<sub>lim</sub>) on the second day. On days three to six, four different interval sessions were performed.

## 2.3 Testing Procedures

Participants were asked to standardize their last meal for all sessions, refrain from alcohol 24hours and caffeinated beverages four hours before testing. Furthermore, participants were instructed not to perform any strenuous activity or strength training the day before testing sessions. The time-of-day for testing was scheduled to be as similar as possible for all sessions across one participant with a total duration of maximal three weeks to complete all tests. The laboratory conditions were held constant ( $19 - 22^{\circ}C$ ), and a fan was placed close to the participant at all times to ensure adequate cooling. Verbal encouragement to the participants was provided to encourage maximal effort whenever necessary, and the participants were generally allowed to listen to self-selected music over a speaker.

#### 2.3.1 Instrumentation

All tests were performed on a Lode Excalibur Sport cycle ergometer (Lode B.V., Groningen, Netherlands) adjusted to the participant's preferences. These preferences were established on the first visit of the participant and replicated for every session. For every test, participants were allowed to choose their preferred cadence. Pulmonary gas exchange and ventilatory parameters were measured using a computerized metabolic system with a mixing chamber (Vyntus CPX, Vyaire Medical GmbH, Hoechberg, Germany). According to the manufacturer's instructions, the system was calibrated before each session, including automated gas calibration with gas concentrations of 15%O2 and 5.85%CO2 (gas from Riessner-Gase GmbH & Co, Lichtenfels, Germany) and automated flow calibration. HR was recorded using a chest strap (Polar H10, Polar Electro OY, Kempele, Finland). Small capillary blood samples were collected from the earlobe to measure lactate (Biosen C-Line, EKF Diagnostics, Barleben/Magdeburg, Germany). Rating of perceived exertion (RPE) was determined using the Borg Scale (6-20), and participants were instructed according to established recommendations.<sup>38</sup>

#### 2.3.2 Experimental protocol

#### 2.3.2.1 Test battery

On arrival on the first day, the participants were weighed, and then a lactate profile test (LT) was performed. The LT started with 150W, increased 25W every fifth minute, and was terminated when a blood lactate concentration of 4mmol·L<sup>-1</sup> was reached. Pulmonary gas exchange, ventilatory parameters, and HR were measured continuously in most tests and all sessions. Lactate and RPE were measured in the last 30 seconds of each step in this test (Figure 2).

Afterward, the participants took an active rest period of ca. 20-minutes. Once a lactate concentration of < 2mmol·L<sup>-1</sup> was achieved, the participants started with a ramp test to determine  $\dot{V}O2_{max}$  and MAP. The test started with one minute at a power corresponding to  $3W\cdot kg^{-1}$  (rounded down to the nearest 50W), increased 25W each minute until exhaustion. Lactate samples were collected right after as well as five and seven minutes after termination of the ramp test (Figure 2). Due to the following lactate minimum test, the participants had to remain passive, being seated without any pedaling or significant body movement.

A modified lactate minimum test (LMT) started seven minutes after finishing the ramp test with 60W below AT 4mmol·L<sup>-1</sup> (rounded to the nearest tenth Watts) and increased 10W every 90-seconds (Figure 2). For this test, no pulmonary gas exchange, ventilatory parameters, and HR were measured. However, lactate samples were taken at the end of each step, and the values were plotted against the workload. The test was terminated once lactate values began to increase again. A schematic overview of test day one is presented in Figure 2.



**Figure 2. Test Day One.** M = Measurement of Lactate and RPE. First, a lactate profile step test (LT) with 25 Watts increments every 5-minute was performed until a lactate concentration of 4mmol·L<sup>-1</sup> was reached. After a ca. 20-minutes rest period, a ramp test to determine maximal oxygen uptake ( $\dot{VO}2_{max}$ ) started. The load increased 25W every minute until exhaustion, and after a 7-minute passive rest, a Lactate minimum test (LMT) concluded the first test day. In this test, the load increased 10W every 90-seconds until the lactate concentration increased again.

The second test day started with an RPE-based warm up protocol modified after Bossi et al.<sup>20</sup>. In order to match the required RPE, participants were allowed to change power on the ergometer accordingly. The first five minutes were done at an RPE of 11, followed by 3x1-minute at RPE 16 with two minutes at RPE 9 in between, except for the last one, which lasted three minutes and was followed by another four minutes at 50% of MAP. The last-minute of the 20-minute warm up was passive rest due to baseline measurements, and the next test started right afterward. This test was a 15-minute AT-effort performed at the power corresponding to lactate minimum, rounded to the nearest five Watts, to determine  $\%\dot{V}O2_{max}@AT$ . Lactate and RPE were measured after 5-,10- and 15-minutes (Figure 3).

After ca. 30-minute active and passive rest,  $T_{lim}$  at MAP was performed. It was ensured that a lactate value below < 2mmol·L<sup>-1</sup> was attained before starting the test. Participants cycled at

their MAP using a freely chosen cadence until exhaustion. The test was terminated when the cadence fell below 60 rpm.<sup>19</sup> A few seconds before the power was set to MAP, the participants were already instructed to pedal in order to attain a suitable cadence once the resistance set in. Lactate was measured directly and 5-minutes after the termination of the test (Figure 3). During the test, no feedback in terms of time or power was provided to the participants, and there was no music allowed. Figure 3 shows the overview of test day two.



**Figure 3. Test Day Two.** M = Measurement of Lactate and RPE. First, participants performed a standardized 20minute warm up based on RPE. It consisted of 5-minutes, followed by 3x1-minute surges with 2-minutes recovery in between (3-minutes after the last surge) and another 4-minutes of easier pedaling. The last-minute was passive rest. After that, participants performed a 15-minutes anaerobic threshold (AT-effort) at a power corresponding to lactate minimum. Every 5-minute, lactate and RPE measurements were taken. The AT-Effort was followed by a ca. 30-minute mixed active (soft-pedaling) and passive (being seated on a chair) rest period. When participants recovered, a time-to-exhaustion test (T<sub>lim</sub>) at maximal-aerobic power (MAP) was performed.

### 2.3.2.2 Interval Sessions

The warm up protocol for all interval sessions was the same as on test day two, and the power used during that first time was replicated for all the following sessions. The two-interval protocols were 3x13x30/15-second intermittent intervals (IM) and 6x5-minutes traditional intervals (TRAD). These protocols were time but not power matched, meaning that one set of 13x30/15-seconds was treated as one interval. For this reason, the 3x13x30/15-seconds equaled 29:15-minutes of interval time and were matched with 6x5-minutes, where the last interval was 4:15-minutes long. The break time in TRAD was set at 2:30-minutes to replicate the protocol used by Bossi et al.<sup>20</sup>, which represents a 2:1 work and rest ratio. The total break time between intervals of 12:30-minutes was also used for the set rest in the IM intervals,

divided into two 6:15-minute blocks. A cool down of 5-minutes was performed right after the last interval. Therefore, both sessions lasted exactly 1-hour and 6:45-minutes. Lactate and RPE were measured at rest, after the warm up and cool down, as well as before and after every interval (Figure 4). The two different interval protocols will be referred to as "types" within the analysis.

As previously done by Bossi et al.<sup>20</sup>, the interval intensity for TRAD in Fixed was set to 84% and 30% of MAP for the recovery periods. The values for IM in Fixed of 100% of MAP for the 30-seconds and 50% of MAP for the 15-seconds were taken from Rønnestad et al.<sup>19</sup>. There were no set breaks in the study protocol from Rønnestad et al.<sup>19</sup>, so those were set at 30% of MAP in this investigation. The participants were only given feedback about the elapsed time and were unaware of their MAP power or power output in these fixed sessions.

In Free, participants were instructed to perform the intervals with a maximal session effort and achieve the best possible average over all intervals. They were informed that a consistent, even pacing from the first to the last interval should be desired. Based on the physiological response during the fixed session, they were given a target value by the investigator. It was, however, emphasized that this was only an approximated target and that they should deviate from that target according to their subjective feeling. It was highlighted that they should use their subjective feeling combined with the gained experience in Fixed to achieve the aim of even and highest average power. The ergometer was set to the same load-restricted mode as in all other sessions. However, participants could manipulate the power output by giving simple hand signs to the investigator, who would then change the power manually. The participants were not blinded to their power in these free sessions to mimic training practice. Fixed and Free will be referred to as "mode" in the analysis.

Approximately 30-minutes after each session, the session's perceived difficulty was assessed using the session RPE (sRPE).<sup>39</sup> An overview of the procedure for the interval sessions is displayed in Figure 4.

10



**Figure 4. Outline interval sessions.** M = Measurement of Lactate and RPE. On each interval session, the standardized 20-minute warm up from day two was performed first. The intermittent interval protocol (IM) consisted of 3x13x30/15-second intervals interspersed with a 6:15-minutes recovery period between sets (A) while the traditional protocol (TRAD) was 6x5-minute intervals (last one only 4:15-minutes) with 2:30-minutes recovery between intervals (B). Both protocols were performed two times - first with a fixed intensity (Fixed; red) based on maximal aerobic power (MAP) and the next time with the maximal session effort as target intensity (Free; green). For Fixed, the IM intervals were performed at 100% (30-seconds)/50% (15-seconds) MAP and the 5-minute intervals of the TRAD at 84% of MAP. The first, second, and third set of IM were compared to the second, fourth, and sixth interval of TRAD, defined as combined time points 1/3, 2/3, and 3/3, respectively. Each session ended with a 5-minute cool down.

## 2.4 Data analysis

For the lactate profile test,  $\dot{V}O2$ , carbon dioxide production ( $\dot{V}CO2$ ), respiratory exchange ratio (RER), and HR were averaged over the last three minutes of each step (Figure 2). Power output at 4mmol·L<sup>-1</sup> (AT 4mmol·L<sup>-1</sup>) was calculated as a linear interpolation of lactate and power in the last two steps.  $\dot{V}O2_{max}$  was calculated as the highest 60-second  $\dot{V}O2$  value from the ramp test and maximal heart rate (HR<sub>max</sub>), maximal breathing frequency ( $f_{Rmax}$ ), and maximal minute ventilation ( $\dot{V}E_{max}$ ) as the highest 30-second value. Peak power output (PPO) was defined as the highest 60-second power during the ramp test. MAP was determined according to the method described by Daniels et al.<sup>40</sup>. This method uses a linear regression from submaximal  $\dot{V}O2$  and power values, in this case, determined during the LT, to extrapolate the power corresponding to  $\dot{V}O2_{max}$ . Following Wahl et al.<sup>41</sup>, power output at lactate minimum (Lac<sub>min</sub>) in the LMT was calculated using the first derivative of a third-order polynomial function placed in the blood lactate vs. workload plot.

During the 15-minutes AT-effort,  $\dot{V}O2$  and HR were averaged only over the last 10-minutes to avoid incorporating low  $\dot{V}O2$  values from the onset of exercise (Figure 3). The  $\%\dot{V}O2_{max}@AT$  was calculated with the 10-minute  $\dot{V}O2$  average of the AT-effort.

For the interval sessions,  $\dot{V}O2$ ,  $f_R$ ,  $\dot{V}E$ , and HR were sampled in 15-second intervals and averaged over each interval and rest period. The power output was also averaged over each interval. To allow for a time point-specific analysis between the two different protocols, the second (1/3), fourth (2/3), as well as the sixth interval (3/3) of the TRAD sessions were compared to the first (1/3), second (2/3), and third interval set (3/3) from the IM sessions, respectively (Figure 4).

#### 2.5 Statistical Analysis

Due to breathing rates exceeding the flow turbine's detection capacity, the data  $f_r$  and ventilation  $\dot{V}E$  of one participant were excluded from the statistical analysis involving these two parameters.

All statistical analyses were performed using the 27<sup>th</sup> version of Statistical Package for the Social Sciences software (SPSS Inc., Chicago, Ill., USA). Data are presented as mean  $\pm$  standard deviation. A two-way repeated-measures ANOVA was performed to investigate the main effects of mode and type as well as interaction effects on t > 90%VO2<sub>max</sub>, t > 90%HR<sub>max</sub>, lactate, HR, power, VE, f<sub>R</sub>, RPE (for all: mean values over the entire interval time), and sRPE. In order to differentiate the variables even more, a three-way repeated-measures ANOVA was performed, adding the time points to the mode and type analysis for lactate, HR, power, VE, f<sub>R</sub>, and RPE. Significant differences in these ANOVA's were analyzed using post hoc tests with a Bonferroni adjustment to identify where the differences existed within the data. Partial eta squared ( $\eta_p^2$ ) was used to compute the strength of associations. Results were adjusted according to the Greenhouse-Geisser correction if the assumption of sphericity was violated. The coefficient of variation (CV) was used to assess between-athlete (inter-individual) variability in t > 90%VO2<sub>max</sub> and t > 90%HR<sub>max</sub> for the different interval sessions.

Linear regression with Pearson's or Spearman's rank correlation coefficient were used to investigate the relationship between t >  $90\%\dot{V}O2_{max}$  in the different interval sessions and  $T_{lim}$  and  $\%\dot{V}O2_{max}@AT$  as well as the relationship between the change of time above t >  $90\%\dot{V}O2_{max}$  and MAP between the exercise modes (Fixed/Free) in the different interval types (IM/TRAD).

The Kolmogorov–Smirnov test was used to test for normality, and the t >  $90\%\dot{V}O2_{max}$  in IM Fixed, TRAD Fixed, and TRAD Free were not normally distributed. Therefore, Spearman's rank coefficient was used for the correlations that involved these parameters. For all other correlations, the Pearson coefficient was used.

A stepwise multiple regression analysis was performed to assess if other physiological variables could significantly predict t >  $90\%\dot{V}O2_{max}$  in the different interval sessions. The level of significance for inferential analyses was set at  $\alpha$  = 0,05.

## 3 Results

The preliminary testing results are presented in Table 3.

| Maximal aerobic power MAP (W)  | $358\pm51$     | [270 – 425]   |
|--|----------------|---------------|
| MAP <sub>rel</sub> (W·kg <sup>-1</sup> )                               | $4.9\pm0.5$    | [4.1 – 5.9]   |
| Anaerobic Threshold AT Lac <sub>min</sub> (W)                          | $295 \pm 49$   | [205 – 355]   |
| Anaerobic Threshold AT Lac <sub>minRel</sub> (W·kg <sup>-1</sup> )     | $4.0\pm0.6$    | [3.2 – 5.3]   |
| Anaerobic Threshold AT 4mmol·L <sup>-1</sup> (W)                       | $299 \pm 51$   | [219 – 372]   |
| AT 4mmol·L <sup>-1</sup> <sub>rel</sub> (W·kg <sup>-1</sup> )          | $4.1\pm0.6$    | [3.4 – 5.5]   |
| %VO2 <sub>max</sub> @AT Lac <sub>min</sub> (%)                         | $85.9 \pm 3.9$ | [79.7 – 91.4] |
| %VO2 <sub>max</sub> @AT 4mmol·L <sup>-1</sup> (%)                      | $85.2\pm4.6$   | [75.6 – 94.3] |
| Time-to-exhaustion at MAP T <sub>lim</sub> (s)                         | $479\pm159$    | [280 – 813]   |
| Maximal heart rate HR <sub>max</sub> (b·min <sup>-1</sup> )            | $192\pm 6$     | [179 – 195]   |
| Maximal breathing frequency f <sub>R</sub> (breath·min <sup>-1</sup> ) | $67.6 \pm 7.4$ | [52.2 – 79.8] |
| Maximal minute ventilation VE (L·min <sup>-1</sup> )                   | $202\pm27$     | [160 – 257]   |

## Table 3. Preliminary testing results

Values are presented as mean  $\pm$  standard deviation and [minimum – maximum]. Anaerobic threshold at lactate minimum (AT Lac<sub>min</sub>), fractional utilization of  $\dot{VO2}_{max}$  at anaerobic threshold lactate minimum ( $\%\dot{VO2}_{max}@AT$ ), fractional utilization of  $\dot{VO2}_{max}$  at anaerobic threshold 4mmol·L<sup>-1</sup> ( $\%\dot{VO2}_{max}@AT$  4mmol·L<sup>-1</sup>)

## 3.1 Comparison between Interval Sessions

The VO2, power output, and VO2 kinetics for the different interval sessions are presented in Figures 5, 6, and 7. A figure combining the VO2 response and power output can be found in the appendices.



**Figure 5. Oxygen uptake (VO2) during the different interval sessions.** Mean VO2 (15-second sampling time) for the intermittent interval session (orange lines) and traditional interval sessions (blue lines). The dashed lines represent the fixed exercise mode, while the solid lines display the free exercise mode. The dashed grey line represents the mean of 90% of VO2<sub>max</sub> for all participants. Note that the area above the grey line does not accurately reflect t > 90%VO2<sub>max</sub> as participants reached 90% of VO2<sub>max</sub> at different time points. For clarity, SD is omitted from the figure.



**Figure 6.** Power output during the different interval sessions. Mean power output for the traditional interval sessions (blue lines) and the intermittent interval session, displayed as each 30/15-second (orange lines) as well as averaged over the whole set (grey lines). The dashed lines represent the fixed exercise mode, while the solid lines display the free exercise mode. For clarity, SD is omitted from the figure.



Figure 7. Time spent at different percentages of  $\dot{VO2}_{max}$  during the interval sessions. The time during the different sessions is displayed as the percentage of total time and plotted against the percentage of  $\dot{VO2}_{max}$ . The orange lines represent the time spent at different percentages of  $\dot{VO2}_{max}$  during the intermittent interval sessions, and the blue lines represent the time for the traditional interval sessions. The dashed lines represent the fixed exercise mode, while the solid lines display the free exercise mode. The values are presented as the mean for each percentage point. For clarity, SD is omitted from the figure.

The free exercise mode (Free) led to greater t > 90% $\dot{V}O2_{max}$ , t > 90%HR<sub>max</sub>, lactate, HR, power, f<sub>R</sub>,  $\dot{V}E$ , RPE, and sRPE compared to the fixed exercise mode (Fixed) in both IM and TRAD (Table 4; all p < 0.001; all  $\eta_p^2$  > 0.795).

A significant main effect of exercise type was found for lactate and power (Table 4; both p < 0.001; both  $\eta_p^2 > 0.686$ ). However, lactate was significantly higher in IM than TRAD (p < 0.01), whereas it was the opposite for the power (p < 0.001).

The t > 90% $\dot{V}O2_{max}$ , was significantly higher in IM than TRAD (Figure 8) for Fixed (p < 0.01) but not for Free (p = 0.321) with a significant main effect on the exercise type ( $\eta_p^2$  = 0.422; p = 0.016). The CV for t > 90% $\dot{V}O2_{max}$  was higher in TRAD Free (30.7%) than in IM Free (18.1%) but lower than in both IM Fixed (61.6%) and TRAD Fixed (89.6%).



**Figure 8. Individual and mean time above 90% VO2**<sub>max</sub>. Displayed for intermittent (IM) and traditional interval session (TRAD) in the fixed (left) and free (right) exercise mode. The black squares display the individual time above 90% VO2<sub>max</sub> in each session. They are connected with a dashed (Fixed) and solid (Free) grey line between the two different interval sessions (IM/TRAD) for each participant. The orange (IM) and blue (TRAD) columns represent the mean time above 90% VO2<sub>max</sub> for each session. \* Indicates a significant difference from IM in the corresponding mode.

No significant difference and was found for t > 90%HR<sub>max</sub> (Figure 9), sRPE, and f<sub>R</sub> between IM and TRAD in neither Fixed nor Free (Table 4). For t > 90%HR<sub>max</sub>, the CV was similar in Free (IM: 36.5%; TRAD: 33.6%) but differed more in Fixed (IM: 106.1%; TRAD: 135.5%).



**Figure 9. Individual and mean time above 90% HR**<sub>max</sub>. Displayed for intermittent (IM) and traditional interval session (TRAD) in the fixed (left) and free (right) exercise mode. The black squares display the individual time above 90% HR<sub>max</sub> in each session. They are connected with a dashed (Fixed) and solid (Free) grey line between the two different interval sessions (IM/TRAD) for each participant. The orange (IM) and blue (TRAD) columns represent the mean time above 90% HR<sub>max</sub> for each session.

Except for the RPE at the first time point in IM (p = 0.139), there was a significant difference between the two modes in both types at the three time-points for all parameters analyzed in the three-way ANOVA (Figure 10).

In the fixed exercise mode, the lactate values during IM were significantly higher compared to TRAD at all time points (all p < 0.05) and the RPE at time point three (p = 0.043). The power, on the other hand, was significantly lower in this mode for IM than TRAD at all time points (all p < 0.001) and HR only at time point three (p = 0.032).

For Free, the HR and power were significantly lower in IM compared to TRAD only at time point one (both p < 0.001), whereas lactate was higher in IM at time point three (p = 0.037). The exact values for all parameters are presented in a supplementary table in the appendices.

Aside from  $\dot{V}E(\eta_p^2 = 0.402; p = 0.027)$  no interaction effect between mode and type was found in the other parameters (Table 4).

| Parameter                 |      | Finad                              | Free   | Mode = Fixed/Free |               | Type = IM/TRAD   |         | Type*Mode  |        |
|---------------------------|------|------------------------------------|--|-------------------|---------------|------------------|---------|------------|--------|
|                           |      | Fixed Free                         |  | $\eta_{p}^{2}$    | р             | η <sub>p</sub> ² | р       | $\eta_p^2$ | р      |
| t > 90%VO2 <sub>max</sub> | IM   | $420\pm259$                        | $1130\pm204^{\text{a}}$                          | 0 017***          | < 0.001       | 0 100*           | 0.016   | 0.076      | 0.262  |
| (s)                       | TRAD | $178\pm159^{b,c}$                  | $1016\pm312^{\text{a,b}}$                        | 0.917             | < 0.001       | 0.422            | 0.010   | 0.070      | 0.302  |
| t > 90%HR <sub>max</sub>  | IM   | $\textbf{416} \pm \textbf{442}$    | $1023\pm374^{\text{a}}$                          | n 797***          | <0.001        | 0.051            | 0 / 59  | 0 291      | 0.057  |
| (s)                       | TRAD | $260\pm352$                        | $1110\pm373^{\text{a}}$                          | 0.757             | <0.001        | 0.031            | 0.455   | 0.291      | 0.037  |
| Lactate                   | IM   | $\textbf{5.83} \pm \textbf{1.86}$  | $8.57\pm2.03^{\text{a}}$                         | 0 705***          | <0.001        | 0 707***         | < 0.001 | 0.049      | 0.469  |
| (mmol·L⁻¹)                | TRAD | $4.68 \pm 1.56^{\text{b,c}}$       | $\textbf{7.73} \pm \textbf{2.19}^{\text{a,b,c}}$ | 0.795             | <b>\U.UUI</b> | 0.707            | < 0.001 | 0.049      | 0.409  |
| Heart Rate                | IM   | $166\pm7$                          | $173\pm6^{\text{a}}$                             | 0 010***          | < 0.001       | 0 / 21*          | 0.017   | 0.047      | 0 21 2 |
| (b∙min⁻¹)                 | TRAD | $163\pm5^{b,c}$                    | $173\pm7^{a,b}$                                  | 0.840 < 0.0       | < 0.001       | 0.421            | 0.017   | 0.047      | 0.512  |
| Power                     | IM   | $296 \pm 42$                       | $316 \pm \mathbf{42^{a}}$                        | 0 010***          | < 0.001       | 0 606***         | < 0.001 | 0.067      | 0 204  |
| (W)                       | TRAD | $300\pm43^{\text{b,c}}$            | $323 \pm 44^{a,b,c}$                             | 0.910             | < 0.001       | 0.000            | < 0.001 | 0.007      | 0.594  |
| f <sub>R</sub>            | IM   | $\textbf{45.8} \pm \textbf{8.3}$   | $51.0\pm6.5^{\text{a}}$                          | 0 006***          | < 0.001       | 0 220            | 0 1 1 4 | 0 272      | 0.001  |
| (breath∙min⁻¹)            | TRAD | $\textbf{42.4} \pm \textbf{4.8}$   | $49.9 \pm 5.9^{\mathrm{a}}$                      | 0.900             | < 0.001       | 0.250            | 0.114   | 0.275      | 0.081  |
| ΫE                        | IM   | $127\pm18$                         | $145\pm16^{\rm a}$                               | 0 062***          | < 0.001       | 0.400*           | 0.010   | 0 402*     | 0.027  |
| (L∙min⁻¹)                 | TRAD | $115\pm10^{b,c}$                   | $141\pm14^{\text{a,b}}$                          | 0.902             | < 0.001       | 0.499            | 0.010   | 0.402      | 0.027  |
| RPE                       | IM   | $\textbf{16.22} \pm \textbf{0.69}$ | $17.56\pm0.70^{\text{a}}$                        | 0 860***          | < 0.001       | 0 178            | 0 150   | 0 220      | 0 106  |
| (Scale 6 – 20)            | TRAD | $15.78\pm0.52^{\circ}$             | $17.61\pm0.45^{\text{a}}$                        | 0.800             | < 0.001       | 0.178            | 0.155   | 0.220      | 0.100  |
| Session RPE               | IM   | $\textbf{6.79} \pm \textbf{0.91}$  | $8.86\pm0.69^{\text{a}}$                         | 0 910***          | <0.001        | 0 188            | 0 283   | 0 190      | 0.280  |
| (Scale 1 – 10)            | TRAD | $\textbf{6.14} \pm \textbf{0.69}$  | $8.79 \pm \mathbf{0.99^a}$                       | 0.910             | <b>\U.UUI</b> | 0.100            | 0.205   | 0.190      | 0.200  |

Table 4. Two-Way ANOVA and post-hoc comparisons for all parameters in IM and TRAD

Values are expressed as mean  $\pm$  standard deviation \* significant factor (p  $\leq$  0.05); \*\*\* significant factor (p  $\leq$  0.001); <sup>a</sup> significantly different from Fixed (p  $\leq$  0.05); <sup>b</sup> significantly different from IM (p  $\leq$  0.05); <sup>c</sup> significantly different from IM at the corresponding mode; Post-hoc comparisons based on Bonferroni's adjustment.

t > 90% VO2<sub>max</sub> = Time above 90% VO2<sub>max</sub>; t > 90% HR<sub>max</sub> = Time above 90% HR; f<sub>R</sub> = breathing frequency; VE = minute ventilation; RPE = Rating of perceived exertion; Session RPE = Perceived exhaustion circa 30min after completing the session



**Figure 10.** Physiological response parameters at the different interval time points. Displayed for lactate, heart rate, power, rating of perceived exertion (RPE), breathing frequency ( $f_R$ ), and minute ventilation ( $\dot{V}E$ ). Values are presented as mean (shapes) and SD (grey lines) either up or down. The Y-axis scale was modified for each variable to ensure the best visibility of the displayed values. Exercise mode: open (Fixed) and filled (Free). Interval type: squares with the blue line (TRAD) and circles with orange line (IM) \* indicate a significant difference between types (IM/TRAD) in the corresponding mode (Fixed/Free) and time point. † indicates a significant difference between modes in both types, and at all time points # indicate significant difference from time point 2/3 in IM at corresponding mode. § indicate significant difference from time point 3/3 in IM at corresponding mode.  $\div$  indicate from time point 3/3 in TRAD at corresponding mode.

## 3.2 Correlation Analysis

No significant correlation was found between %VO2<sub>max</sub>@AT and t > 90%VO2<sub>max</sub> in IM Fixed (p = 0.191), IM Free (p = 0.857), TRAD Fixed (p = 0.273), or TRAD Free (p = 0.527) (Figure 11).



**Figure 11. Relationship between %** $VO2_{max}$ **@AT and t > 90%**  $VO2_{max}$ . Correlation of fractional utilization of  $VO2_{max}$  at anaerobic threshold (% $VO2_{max}$ @AT) and time above 90%  $VO2_{max}$  (t > 90%  $VO2_{max}$ ) in the different interval sessions. Exercise mode: open (Fixed) and filled (Free). Interval type: squares with blue regression line (TRAD) and circles with orange regression line (IM). The regression equation and correlation coefficient (r) are displayed on the graph for each correlation calculation.

Also, no significant correlation between t > 90%VO2<sub>max</sub> in any of the sessions and T<sub>lim</sub> was found (Figure 12; IM Fixed: p = 0.649; IM Free: p = 0.121; TRAD Fixed: p = 0.616 and TRAD Free p = 0.681).

There was a significant correlation between change of t > 90%  $\dot{V}O2_{max}$  and MAP in percent from Fixed to Free for IM (p < 0.05) but not for TRAD (p = 0.295) (Figure 13).

No physiological parameter in the multiple regression analysis could significantly predict t >  $90\%\dot{V}O2_{max}$  in more than one interval session.



**Figure 12. Relationship between T**<sub>lim</sub> and t > 90%  $\dot{VO2}_{max}$ . Correlation of time-to-exhaustion at maximal aerobic power (T<sub>lim</sub>) and time above 90%  $\dot{VO2}_{max}$  (t > 90%  $\dot{VO2}_{max}$ ) in the different interval sessions. Exercise mode: open (Fixed) and filled (Free). Interval type: squares with blue regression line (TRAD) and circles with orange regression line (IM). The regression equation and correlation coefficient (r) are displayed on the graph for each correlation calculation.



**Figure 13. Relationship between the change of t > 90% VO2<sub>max</sub> and MAP.** Correlation of the change of time above 90% VO2<sub>max</sub> (t > 90% VO2<sub>max</sub>) and maximal aerobic power (MAP) between the exercise modes (Fixed/Free) in the different interval types. The dashed orange line (left) represents the relationship for the intermittent interval sessions (IM), and the dashed blue line (right) represents the relationship for the traditional interval sessions (TRAD). The displayed values are the calculated difference between t > 90% VO2<sub>max</sub> in Fixed and Free (individual t > 90% VO2<sub>max</sub> from the Free session minus the individual t > 90% VO2<sub>max</sub> from the Fixed session) and between the change in power output presented as percentage of MAP (individual average power output from the Free session minus the individual average power output from the Fixed session) for each interval type (IM left; TRAD right). The regression equation and correlation coefficient (r) are displayed on the graph for each correlation calculation. \* indicates significance p < 0.05

## 4 Discussion

Due to the high inter-individual variability observed in studies investigating t >  $90\%\dot{V}O2_{max}$ , this study aimed at investigating the influence on this variability between different interval protocols and modes of intensity prescription. In addition, the physiological response of a classic 3x13x30/15-second intermittent interval protocol was compared to a traditional interval protocol of 6x5-minutes instead of 4x5-minutes.

The main findings of this study included that neither of the physiological parameters  $\%\dot{V}O2_{max}@AT$  nor  $T_{lim}$  was correlated with t > 90 $\%\dot{V}O2_{max}$  in any of the sessions. The CVs for t > 90 $\%\dot{V}O2_{max}$ , however, were nearly three times higher in the fixed exercise mode (Fixed) compared to the maximal session effort approach (Free), indicating a methodological influence instead. There was also a higher response across all parameters in Free than Fixed regardless of the exercise type (IM/TRAD), revealing that the intensity during Fixed was considerably easier. Furthermore, the physiological response between IM and TRAD in Free was very similar as t > 90 $\%\dot{V}O2_{max}$  was not different. Also, in Free, lactate, HR, and RPE were different only at one out of three time points when comparing between the two exercise types.

### 4.1 Physiological Factors

Contrary to our hypothesis and previous suggestions<sup>28</sup> there was no correlation for  $\%\dot{V}O2_{max}@AT$  and  $T_{lim}$  with t > 90 $\%\dot{V}O2_{max}$ . It seemed conceivable that a higher  $\%\dot{V}O2_{max}@AT$  increased t > 90 $\%\dot{V}O2_{max}$  because the necessary intensity would be easier to sustain. However, not even the participant who had a  $\%\dot{V}O2_{max}@AT$  above 90% achieved the highest t > 90 $\%\dot{V}O2_{max}$  in any of the sessions, highlighting the physiological demands between continuous and interval exercise might be different.

This difference is further supported by the observation that the duration of  $T_{lim}$  was not associated with > 90% $\dot{V}O2_{max}$ . Previous investigations with  $T_{lim}$  at  $v\dot{V}O2_{max}$  indicated that the AT, presented as a percentage of  $v\dot{V}O2_{max}$ , correlates positively with  $T_{lim}^{42,43}$  because runners with a bigger difference between AT and  $v\dot{V}O2_{max}$  had a shorter  $T_{lim}$ .<sup>42</sup> It seemed possible that a shorter  $T_{lim}$  would also be associated with a reduced t > 90% $\dot{V}O2_{max}$  because metabolites would build up more rapidly during interval sessions and prevent participants from completing the session with the fixed intensity or from producing the necessary intensity in the free exercise mode. However, not even the relationship between AT and  $T_{lim}$  could be confirmed with our data. This is surprising but might be because of the modality differences between cycling and running.

Another factor that challenges identifying a link between the parameters above and t >  $90\%\dot{V}O2_{max}$  could be intra-individual differences in the test results. In their perspective piece, Chrzanowski-Smith et al.<sup>44</sup> recommend repeated testing as a possible solution but also acknowledge the lacking feasibility of such procedure in scientific practice. Within this investigation, we did not repeat tests but validated  $\%\dot{V}O2_{max}@AT$  with an additional trial and replicated tests as well as session designs from related studies to ensure comparability. It might be argued that conducting  $T_{lim}$  after the AT-effort impaired performance, but this is unlikely because the time was even 1,5-minutes longer than previously reported.<sup>45</sup> In the study from Bossi et al.<sup>20</sup> MAP was 0.27W·kg<sup>-1</sup> higher than in our study, possibly also explaining the longer  $T_{lim}$ . The reason for the lower MAP in our investigation is unclear as the same methodology for MAP determination was used. But even though the characteristics of the participants in those two studies<sup>20,45</sup> and ours are also similar, it is elusive to draw meaningful conclusions from these comparisons as differences in the equipment and general settings could still exist.

In fact, the finding that none of the many other physiological parameters assessed in this study could predict t >  $90\%\dot{V}O2_{max}$  in more than one interval session makes a physiological influence unlikely as a primary cause of the individual differences.

## 4.2 Methodological Factors

In the present investigation, the participants were able to exercise at a higher intensity in the free exercise mode, which is mirrored in a greater physiological response demonstrated across all parameters. This finding combined with different magnitudes of change between Fixed and Free in individual participants could indicate inadequate intensity matching and subsequently individual variation in t > 90%VO2<sub>max</sub> when using fixed percentages of MAP. In order to compare interval protocols, various ways of matching and normalizing intensity have been used, but many have recently faced criticism.<sup>30,31</sup> Although MAP itself was not analyzed in the

extensive review by Jamnick et al.<sup>31</sup> especially fixed percentages of maximal anchors like  $\dot{V}O2_{max}$  or  $W_{max}$  evidently have "substantial shortcomings as a means for normalizing exercise intensity between individuals."<sup>31,p.11</sup> The lack in normalizing intensity between individuals when using percentages of MAP was also emphasized by the comparison of the change in work rate and t > 90% $\dot{V}O2_{max}$  from Fixed to Free in our investigation.

While every participant was able to work at a higher intensity in Free, the actual degree was individual. In IM, this degree of a greater work rate is positively correlated to the change in t > 90%VO2<sub>max</sub> between the two modes. Although this correlation was not found in TRAD, possibly influenced by three athletes unable to maintain power because they started too hard, it emphasizes that the fixed intensity was in relative terms easier for some athletes. As far as we are aware, no other studies have directly compared a fixed and free intensity prescription, but the heterogeneous metabolic perturbations following fixed approaches have been demonstrated before.<sup>30–32</sup> Iannetta et al.<sup>30</sup> demonstrated that in a large, heterogeneous group of 100 untrained and well-trained individuals, the maximal lactate steady state (MLSS) occurred at a wide range if quantified as percentages of VO2<sub>max</sub>, W<sub>max</sub>, and HF<sub>max</sub>. This wide range affirms that the location of commonly used exercise domains (moderate, heavy, and severe) can be highly variable between individuals when prescribed as percentages of maximal values. For example, in our investigation, %VO2<sub>max</sub>@AT also occurred at a wide range of ~ 75 – 95%. So, 85% of  $VO2_{max}$  would be heavy exercise for some, while it would be severe for the other, likely resulting in a different metabolic strain and individual differences in the acute physiological response such as t >  $90\%\dot{V}O2_{max}$ .

Especially in studies comparing various interval protocols using fixed percentages of MAP<sup>20</sup> or maximal aerobic speed<sup>28</sup> large individual differences in t > 90% $\dot{V}O2_{max}$  have been observed. This vast variation was also present in the current investigation for both types of exercise. Nonetheless, a direct comparison of the CV between the two modes of exercise prescription shows that the CV is nearly three times higher in Fixed regardless of the interval protocol, possibly strengthening the cause of a methodological reason for the disparity in t > 90% $\dot{V}O2_{max}$ . As the CV represents the magnitude of the standard deviation to the mean, it is, of course, influenced by long or short mean t > 90% $\dot{V}O2_{max}$ . O'Grady et al.<sup>33</sup>, who also used the maximal session effort, presented a CV between athletes of over 200% for IM due to only 140-seconds t > 90% $\dot{V}O2_{max}$ . However, their IM sets were 20-minutes long, likely making it difficult to maintain an intensity high enough to elicit substantial t > 90% $\dot{V}O2_{max}$ . The standard deviation for IM Free in our study, on the other hand, was nearly a minute less compared to IM Fixed, even though the mean was much higher for IM Free. Moreover, when visually inspecting the individual distribution of t > 90% $\dot{V}O2_{max}$  and discounting some outliers due to pacing problematics, it becomes clear that the range of variation is around 6-minutes across both modes and types despite mean t > 90% $\dot{V}O2_{max}$  being over 10-minutes more in Free.

The latter is, of course, rather an observational finding and is influenced by our specific results in Fixed. It is noteworthy that compared to the results by Bossi et al.<sup>20</sup>, where the fixed TRAD protocol was identical, and the participants' characteristics similar, the t > 90% $\dot{V}O2_{max}$  was around 40% (1,5-minutes) less compared to our values with the standard deviation being equal. The total t > 90% $\dot{V}O2_{max}$  was still below 5-minutes and the CV close to 60%, which is in comparison to results in our study one third lower but still double the CV of TRAD Free. This variety might be related to the slight differences in MAP between studies.

In general, the more similar physiological response between Free but not Fixed in our study highlights the necessity to prescribe intensity adequately but the comparison between IM and TRAD emphasizes the same regarding time matching.

## 4.3 Comparison IM and TRAD

Identifying the most effective interval design has been one area of research in sports science over the last decades. IM protocols showed to elicit a high physiological response as they allow to exercise at relatively high intensities compared to longer aerobic intervals.<sup>19</sup> But IM are in practice also performed in a stacked manner, meaning that multiple high-intensity repetitions are interspersed only with very short recovery periods and grouped as an interval set. To the best of our knowledge, this is the first study where these sets were treated as one interval so that the total interval time of the 3x13x30/15-seconds protocol equaled 29:15-minutes, and the power output was averaged over the whole set instead of only accounting for the 30-seconds high intensity.

Due to the averaging of the power, it became apparent that even though the power in the free exercise mode was significantly higher in TRAD compared to IM, the difference in absolute terms was only 7W. This absolute difference could even be lower because the constant adjustment in power during IM led to values being ca. 2 - 3W below the actual target

values. The small difference could indicate that a time matching in this form may be appropriate. However, direct comparisons with similar investigations are difficult because previously, just the 30-seconds were considered, resulting in differences of 30 - 50W between IM and longer intervals.<sup>29,46</sup> It seems that when the overall interval and break time are identical, the intensity chosen by the athlete using the maximal session effort is quite similar despite a variation in the interval protocol.<sup>47</sup>

Comparing the physiological response to the two protocols also implies a more similar outcome within this format of time matching. In Free, the lactate values were higher for IM than TRAD, while none of the other parameters differed. These higher values are in contrast to previous studies from Almquist et al.<sup>29</sup> and Rønnestad et al.<sup>46</sup> where lactate was not different between IM and 4x5-minutes interval sessions.<sup>29,46</sup> The reason for the higher lactate values in this study could be due to the power surge in the last two 30-second repeats at the end of the IM set as the time point-specific analysis showed that the values were only significantly different at the last time point. In addition, Almquist et al.<sup>29</sup> also used the maximal session effort and reported differences between the sessions in t >  $90\%\dot{V}O2_{max}$  and t > 90%HR<sub>max</sub>. These are considered two crucial variables in quantifying the effectiveness of interval sessions<sup>21,26,27</sup> and no differences between them were found in the present study. The t > 90%VO2<sub>max</sub> tended to be higher in IM, but that observation might be influenced by three participants who achieved a lot less t > 90%VO2<sub>max</sub> in TRAD. A reason for this could have been the pacing in TRAD because some participants started too strong, subsequently dropping in power and  $\dot{V}O2$ , not able to "fully" recover from it again. T > 90%HR<sub>max</sub>, on the other hand, generally had a substantial inter-individual variability, but as there was no difference between IM and TRAD, the physiological response seems to be similar.

The physiological rationale of using IM is to exercise at higher intensities for a longer time and taxing the cardiovascular system to a very high degree but without accumulating the same amount of fatigue as in continuous exercise at the same intensity.<sup>17,19,48</sup> This is achieved by incorporating short rest periods in a ratio of 2:1 to the work interval because "doubling the recovery periods [...] contributes to the fall in HR and VO2 during the recovery thereby delaying time to achieve > 90% $VO2_{max}$  during the work intervals".<sup>19,p.1003</sup> Figure 5 shows the VO2 is not really dropping but continuously rising throughout each set. Longer aerobic intervals are designed for the same purpose yet need to be performed with a much lower

intensity since the single interval duration is longer. The lower intensity is still high enough to elicit  $90\%\dot{V}O2_{max}$ , as also notable in Figure 5, but the overall time becomes an important factor, making time-matching very important.

Regarding session design, the only major distinction between this study and the one from Almquist et al.<sup>29</sup> is the total interval time for TRAD, which was ca. one third longer in this investigation. The maximal session effort requires athletes to go as hard as possible, independent of work duration, thereby causing them to "adjust their intensity such that blood lactate and perceived exertion responses throughout each session are essentially identical".<sup>49,p.321</sup> When using self-paced intervals, multiple other studies also showed that the RPE follows a nearly linear increase, making comparisons based on time-independent variables complicated while highlighting the importance of adequate time-matching. In an investigation by Fennell and Hopker<sup>47</sup>, who did not directly compare t > 90% $VO2_{max}$  because their main objective was the influence of break intensity, the t > 90% $VO2_{max}$ , and t > 90%HR<sub>max</sub> are not different between IM and TRAD in this investigation could strengthen the notion that integrating the 15-seconds into the total interval time of an IM protocol is more accurate because only accounting for the work periods of 30-seconds is not reflecting the acute cardiovascular response.

Interestingly there were more differences between IM and TRAD in the fixed exercise mode, with a higher physiological response in IM for some of the key variables like t > 90% $\dot{V}O2_{max}$ , lactate, HR, and RPE. Nevertheless, the difference of 4W in average power (1.5% in MAP) during the intervals was less than the one observed in Free. One argument could be that the averaging of the IM intervals is not accurately reflecting the physiological strain but the homogenous response in Free disputes that. It could also indicate that if the intensity is not completely maximal, the longer intervals and their cardiovascular training effect are underestimated. This observation may appear false as, especially lately, more and more designs with varying intensity have emerged, showing to elicit higher t > 90% $\dot{V}O2_{max}$  than longer intervals. In a very recent investigation, Beltrami et al.<sup>50</sup>, also using maximal effort matching, found that an interval protocol in which the power was decreased during an interval (DEC) elicited a higher t > 90% $\dot{V}O2_{max}$  was due to excess  $\dot{V}O2$  accumulated in the first

phase of the DEC interval maybe caused by the loss of efficiency due to the recruitment of more type 2 fibers or fatigue.<sup>50</sup> These factors are rather related to a stimulus on the muscularand not the cardiovascular level, potentially reducing the importance of a higher t >  $90\%\dot{V}O2_{max}$  for these protocols. Instead, it could be assumed that the benefits of these mixed protocols with periods of very high intensities like DEC and IM are especially within the periphery involving specific muscle recruitment and lactate removal.<sup>17</sup> Both areas are eminently triggered within IM, which was notably indicated for the muscle recruitment by the improvements of  $W_{max}$  and the power in a Wingate test following a 10-week intervention.<sup>46</sup> We did not assess anaerobic contribution, but because of the higher power of IM when only considering the 30-second intervals, greater involvement can be assumed. The alternating nature of IM and its possible benefits for lactate removal might be more relevant for sports like cycling, which also have a more intermittent requirement profile.

These considerations beyond the seemingly pure cardiovascular stimulus of t >  $90\%\dot{V}O2_{max}$ , combined with our finding of a more uniform physiological response between IM and TRAD, may highlight the need to reduce controversy around the effectiveness of different interval protocols.

## 4.4 Strength, Limitations, and Future Perspective

Regarding the maximal session effort, it is important to note that the participants were given a target by the investigator and were cycling in a load-restricted mode on a power they were not blinded to. Even though comparable studies usually use a non-target and blinded approach, it needs to be emphasized that in training practice, athletes typically also have targets based on previous experience and are aware of their power throughout the session. However, especially in TRAD, constant pacing seemed to be more complex, and thus participants are required to have experience with HIIT as well as good abilities for pacing. Future studies should also consider performing multiple sessions to clearly identify the constant best average for the individual participant.

Another limitation that needs to be mentioned is the higher break intensity for TRAD in the free exercise mode. The intensity was set to half of the interval power due to technicalities of the equipment instead of 30% of MAP as in the other sessions. Participants were also able to adjust the power in the breaks but generally did not. As visible in Figure 7, where the lines for

the time at a given % of  $\dot{V}O2_{max}$  are overlapping from 80% onwards again, it had likely no influence on the physiological response in the higher intensities. This observation is supported by Fennell and Hopker<sup>47</sup>, who also used the maximal session effort and did not find differences in t > 90% $\dot{V}O2_{max}$  when comparing various break intensities.

Generally, the interval protocols in the present investigation were exactly replicated from previous studies, which ensures comparability and allows for valid classification of the findings among the existing literature. Additionally, the thorough determination of  $\%\dot{V}O2_{max}@AT$  with using the lactate minimum test and the 15-minute threshold effort to identify the "true" fractional utilization as one of the main parameters is another strength of our study.

These thoughtful considerations and the potentially minor influence of individual physiology on t > 90% $\dot{V}O2_{max}$  even in Free despite some variation in t > 90% $\dot{V}O2_{max}$  also in those sessions, indicate the importance of methodological aspects and should be further investigated. The maximal session effort seems to be a valid method to standardize intensity, at least in welltrained athletes. Identifying a true constant best average may equalize the physiological response even more. Moreover, the day-to-day variation and acute training status could influence the determination of  $\dot{V}O2_{max}$  and  $HR_{max}$ , subsequently leading to over-/underestimation of the outcome variables from the interval sessions. Therefore, future investigations should consider using a pre-phase where training is already standardized and multiple max-tests are performed. This rigorous standardization might allow to more accurately identify whether individual variation in the acute physiological response following an interval session is only due to methodology.

## 4.5 Conclusion

The findings of our study indicate that large individual differences observed in t > 90% $\dot{V}O2_{max}$  across different interval protocols seem to be caused by methodological considerations rather than physiological parameters. No physiological variable assessed in this study was correlated to t > 90% $\dot{V}O2_{max}$ . Instead, the variation was reduced when the sessions were performed with the maximal session effort and not with fixed intensities. The intensities based on MAP elicited a different physiological response between athletes because they were easier for some than others. When regulated by the athletes, the intensity was much higher and led to a bigger physiological strain. From an applied perspective, it may therefore be beneficial to focus on

executing the maximal session effort well instead of using fixed values to get the greatest training response.

Additionally, t > 90% $\dot{V}O2_{max}$  and t > 90%HR<sub>max</sub> as important parameters in HIIT were similar for IM and TRAD in Free, indicating that the short rest periods in intermittent interval sets should be included when time-matched with longer intervals to better reflect the cardiovascular stimulus. Thus, for improving  $\dot{V}O2_{max}$ , the appropriate time-matching and intensity prescription could be more important than the interval design.

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## Appendices

| Daramatar                    | Mada          | Turno | Interval time point                                       |   |  |  |
|------------------------------|---------------|-------|---|---|--|--|
| Parameter                    | moue          | Type  | 1/3   | 2/3   | 3/4  |  |
|                              | Fixed         | IM    | $5.45 \pm 1.64^{\text{tms}}$                              | $5.91 \pm 1.89^{\dagger}$                                 | $\textbf{6.14} \pm \textbf{2.09}^{\dagger}$              |  |
| Lactate                      | FIXEU         | TRAD  | $\textbf{4.66} \pm \textbf{1.52}^{\texttt{+}} \textbf{*}$ | $\textbf{4.96} \pm \textbf{1.57}^{\texttt{+}} \textbf{*}$ | $\textbf{4.87} \pm \textbf{1.86}^{\texttt{+}}\textbf{*}$ |  |
| [mmol·L <sup>-1</sup> ]      | Free          | IM    | $6.85\pm1.55^{\text{\#}\S}$                               | $8.81\pm2.00^{\S}$  | $10.0\pm2.75$  |  |
|                              | FIEE          | TRAD  | $6.87 \pm 1.84^{+ \ddagger}$                              | $\textbf{8.39} \pm \textbf{2.40}$                         | $9.08\pm3.06^{\ast}$                                     |  |
|                              |               |       |   |   |  |  |
|                              | Fixed         | IM    | $163\pm7^{^{+}\#\$}$                                      | $167\pm7^{^{+}\$}$  | $169\pm7^{\dagger}$                                      |  |
| Heart Rate                   | TIXEU         | TRAD  | $162\pm5^{\text{+++}}$                                    | $165\pm5^{\rm +\pm}$                                      | $\textbf{166} \pm \textbf{5^{**}}$                       |  |
| [b·min⁻¹]                    | Free          | IM    | $168\pm\mathbf{6^{\#\$}}$                                 | $174\pm6^{\$}$  | $178\pm 6$   |  |
|                              | Free          | TRAD  | $171\pm7^{\pm **}$  | $175\pm7^{\ddagger}$                                      | $\textbf{178} \pm \textbf{7}$                            |  |
|                              |               |       |   |   |  |  |
|                              | Fixed         | IM    | $296 \pm \mathbf{42^{+}}$                                 | $296\pm42^{\dagger}$                                      | $296\pm42^{+}$   |  |
| Power                        | Плец          | TRAD  | $300\pm43^{**}$   | $300 \pm 43^{+*}$   | $300 \pm 43^{+*}$  |  |
| [W]                          | Free          | IM    | $312\pm42^{\text{\#}\S}$                                  | $318 \pm 43$  | $318 \pm 43$   |  |
|                              | 1166          | TRAD  | $325 \pm 44^{+*}$   | $320 \pm 44$  | $324 \pm 46$   |  |
|                              |               |       | +#6   | +c  | <b>.</b>   |  |
|                              | Fixed<br>Free | IM    | 43.0 ± 7.9 <sup>+#9</sup>                                 | 46.3 ± 8.8 <sup>19</sup>                                  | $48.4 \pm 8.4^{+}$                                       |  |
| f <sub>R</sub>               |               | TRAD  | $41.5 \pm 5.1^{++}$                                       | $43.2 \pm 5.1^{+}$  | $44.6\pm4.6^{+}$   |  |
| [breath·min <sup>-1</sup> ]  |               | IM    | $46.7 \pm 7.2^{\#\$}$                                     | $51.0\pm6,4^{\$}$   | $55.3\pm6.4$   |  |
|                              |               | TRAD  | $46.6 \pm 6.4^{+\pm}$                                     | $51.5\pm6.0^{\rm \ddagger}$                               | $56.2 \pm 5.9$   |  |
|                              |               | IM    | 1 <b>71 + 18<sup>†#§</sup>*</b>                           | 128 + 19 <sup>†§</sup> *                                  | 132 + 10†*   |  |
| ŃЕ                           | Fixed         | TRAD  | $121 \pm 10$<br>$114 \pm 11^{+++}$                        | $120 \pm 10^{+10^{+10^{+10^{+10^{+10^{+10^{+10^{+$        | $132 \pm 10^{+10}$                                       |  |
| v∟<br>[I∙min <sup>-1</sup> ] |               | IM    | $134 + 16^{\#\$}$   | $110 \pm 10$  | 155 + 17   |  |
|                              | Free          |       | $134 \pm 10$  | $147 \pm 17$<br>$146 \pm 17$                              | $155 \pm 17$<br>$152 \pm 16$                             |  |
|                              |               | INAD  | 137 ± 13  | 140 ± 14  | $152 \pm 10$   |  |
|                              | Five -        | IM    | $15.58\pm0.67^{\$}$                                       | $\textbf{16.25} \pm \textbf{0.86}^{\texttt{+}}$           | $\textbf{16.83} \pm \textbf{1.11}^{\dagger}$             |  |
| RPE                          | Fixed         | TRAD  | $\textbf{15.58} \pm \textbf{0.67}^{\dagger}$              | $\textbf{15.92} \pm \textbf{0.67}^{\dagger}$              | $16.08\pm0.67^{*}*$                                      |  |
| [Scale 6 – 20]               |               | IM    | $16.08 \pm 0.90^{\text{\#}\S}$                            | $17.67\pm0.89^{\S}$                                       | $18.92\pm0.73$   |  |
|                              | Free          | TRAD  | $16.58 \pm 0.67^{++}$                                     | $\textbf{18.00} \pm \textbf{0.60}^{\ddagger}$             | $19.00\pm0.96$   |  |

## Table. Post hoc comparisons for all parameters analyzed in the three-way ANOVA

Values are presented as mean  $\pm$  standard deviation.  $f_R$  = breathing frequency,  $\dot{V}E$  = minute ventilation, RPE = rating of perceived exertion (Borg Scale)

\* indicate a significant difference between types (IM/TRAD) in the corresponding mode (Fixed/Free) and time point. † indicates a significant difference between modes in both types, and at all time points # indicate significant difference from time point 2/3 in IM at corresponding mode. § indicate significant difference from time point 3/3 in IM at corresponding mode. + indicate significant difference from time point 2/3 in TRAD at corresponding mode. \$ indicate significant difference from time point 2/3 in TRAD at corresponding mode. + indicate significant difference from time point 3/3 in TRAD at corresponding mode.



Figure. Power Output and VO2 response during the different interval sessions in the fixed (top) and free exercise mode (bottom). Mean VO2 with a 15-second sampling time (dashed lines) and power output (solid lines) for the traditional interval sessions (blue lines), and the intermittent interval session displayed as averaged over the whole set (orange lines) as well as each 30/15-second repetition (grey lines). The solid black line represents the mean of 90% of VO2<sub>max</sub> for all participants. Note that the area above the black line does not accurately reflect t > 90%VO2<sub>max</sub> as participants reached 90% of VO2<sub>max</sub> at different time points. For clarity, SD is omitted from the figure.

### Athlete Questionnaire

ID/Name: \_\_\_\_\_

1. Which sports are you or have you been performing in?

How long are you/ have you been doing these Sports (list sports and years)

| Sport | Years |
|-------|-------|
|       |       |
|       |       |
|       |       |
|       |       |
|       |       |

- 2. What is your main sport now?
- 3. Did you have any major injuries?

How long did you need to pause training?

Anything in the past 6 months?

Considering the last 6 month

- 4. How many hours of endurance training are you doing? (per week)
- 5. How many hours of strength training (gym/ heavy lifting) are you doing? (per week)
- 6. How many hours per week do you train in your main sport? (if triathlon, specify hours per discipline per week)
- 7. If not already stated, how many hours of cycling training are you doing? (per week)
- 8. How often do you perform interval sessions/ high intensity training on the bike?

On a scale from 1 to 10 (1 being the lowest)

- 9. How would you rate your current fitness level? (just compared to yourself)
- 10. How experienced are you with high-intensity interval training? (VO2max sessions)
- 11. How experienced are you with short/ intermittent interval sessions specifically? (30/15s; 40/20s; 1min on/off etc.)

Racing

12. Have you participated in bike races? (specify sub disciplines e.g. Road, MTB, Cross etc.)

How many? (approximately)

How many this season?

How experienced are you in bike racing? (scale from 1 to 10)

13. Which of the following rider types would you consider yourself? (cross the best fitting one)

- Sprinter
- Climber
- TT-Specialist
- Puncheur
- Rouleur



