



## A Short Maximal Effort Induces Greater Low-Frequency Fatigue than Prolonged Cycling in Highly-Trained Road Cyclists

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### Abstract

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This study aimed to investigate the sensitivity of low-frequency fatigue (LFF) in high-level road cyclists following two distinct cycling efforts: a 30-minute maximal effort (30'TT) and a 4-minute maximal effort (4'TT). Twenty-one participants were included in this study (age:  $22.8 \pm 4.8$  years; height:  $169.9 \pm 3.5$  cm; body mass:  $68.5 \pm 8.5$  kg), and LFF, perceived fatigue and muscle soreness were monitored at baseline, 30 minutes and 24 hours post-fatigue protocol. Linear mixed model analysis was employed to assess the changes caused by protocol, time, and limb dominance, and a repeated-measures correlation was used to assess the association between LFF and perceived fatigue or muscle soreness. Significant fatigue was induced by both protocols ( $p < 0.001$ ), with LFF scores returning to baseline after 24 hours. Notably, following the fatigue protocols, a significantly lower LFF score was observed after the 4'TT compared to the 30'TT ( $d = 0.39$ ,  $p = 0.032$ ). Furthermore, a large and significant association between LFF scores and perceived fatigue ( $rm_{corr} = -0.5$ ,  $p < 0.001$ ) was identified, as well as a weak and significant association between LFF and perceived muscle soreness ( $rm_{corr} = -0.28$ ,  $p < 0.001$ ). Taken together, these findings seem to support LFF as a sensitive, field-based tool for monitoring acute peripheral fatigue following cycling efforts.

Keywords: Sports, athletic Performance, fatigability, exercise Intensity

### Introduction

Physical fatigue is a complex physiological phenomenon that occurs during and after intense or prolonged physical activity, playing a crucial role in sports performance.<sup>1</sup> While it interacts with various systems in the body (e.g., musculoskeletal, cardiovascular, and central nervous system), and is seen as a natural response to exercise, it can lead to different adaptations depending

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on the frequency and severity experienced by athletes.<sup>2,3</sup> Fatigue is essential for improving performance, when appropriately dosed and followed by proper recovery.<sup>3</sup> However, when an excessive stimulus is applied, especially if followed by inadequate recovery, fatigue can persist and lead to decreased performance – ultimately resulting in non-functional overreaching and overtraining – as well as increased risk of injury.<sup>4,5</sup>

In this sense, the importance of monitoring athletes' physical fatigue is recognized by coaches and their supportive staff.<sup>6</sup> In road cycling, fatigue is commonly assessed using a combination of physiological, performance-based, and subjective measures. The response induced by exercise is typically monitored through various physiological markers, including, but not limited to, changes in neuromuscular function (e.g., maximal voluntary contraction, jump performance)<sup>7</sup>, biochemical markers (e.g., creatine kinase, interleukin-6)<sup>8</sup>, physiological parameters (e.g., heart rate and blood lactate concentration)<sup>9,10</sup>, and subjective perceptions (e.g., rating of fatigue scale, visual analogue scales).<sup>11</sup> Although not all these markers directly indicate fatigue, they might offer valuable insights into athletes' physiological status and readiness to perform, helping to adjust training loads and optimize the recovery process.<sup>12</sup> Nevertheless, identifying the most sensitive and reliable methods that can effectively track fatigue remains challenging both in both amateur and elite settings.<sup>13</sup>

A technology that is gaining renewed attention is the measurement of low-frequency fatigue (LFF).<sup>14</sup> Although earlier studies on this type of fatigue exist, they were largely confined to controlled laboratory settings.<sup>15</sup> More recently, however, a new technology has been developed and introduced to the market (Myocene device, Liège, Belgium), enabling the assessment of LFF in field conditions.<sup>16</sup> This advancement is possible due to the technology's portability and the relatively quick and easy nature of the measurements.<sup>16</sup> It has been reported that LFF is a persistent form of muscle fatigue, marked by a significant reduction in force production at lower stimulation frequencies (10–20 Hz) compared to higher frequencies (50–100 Hz).<sup>16,17</sup> Indeed, previous research suggests that LFF is associated with a decrease in muscular contractile activation, which may result from a failure in excitation-contraction coupling. This failure is thought to be caused by a reduction in calcium ( $\text{Ca}^{2+}$ ) release from the sarcoplasmic reticulum.<sup>15,17</sup> In this context, assessing post-exercise peripheral changes through electrical stimulation of the muscles could offer valuable insights into the efficiency of signal transmission from sarcolemma excitation to the binding of  $\text{Ca}^{2+}$  to contractile proteins, ultimately providing objective data on athletes' physical fatigue states.<sup>18,19</sup>

The manifestation of LFF across different efforts remains to be studied. While previous research has explored it following different efforts, such as after eccentric exercise or soccer matches<sup>14,16</sup>, scientific evidence regarding LFF assessments remains limited at an early stage, highlighting the need for further exploration. One key aspect that might require clarification is understanding how LFF responds to different types of efforts, specifically how short, high-intensity efforts compare to longer, sustained efforts, in relation to neuromuscular status assessed via LFF. In this sense, the present study aims to address this gap by assessing LFF following different cycling efforts, with the goal of identifying characteristic patterns of LFF. Specifically, we aim to determine which type of effort – prolonged versus short high-intensity – is more likely to elicit substantial changes in LFF responses. We hypothesize that short, high-intensity efforts will induce more pronounced LFF compared with longer, sustained efforts, as they are more peripherally demanding, relying on greater local muscle activation, whereas longer efforts may elicit a relatively greater central fatigue due to prolonged motor drive and neural adjustments.

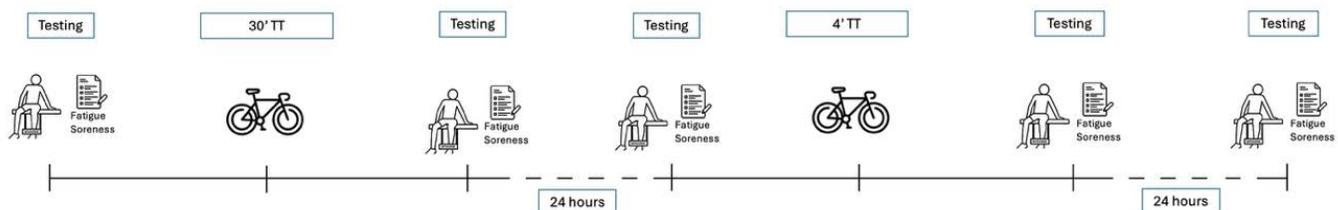
## Methods

### Participants

Twenty-one male road cyclists, familiarized with time-trial efforts were included in this study (tier 3 according to McKay et al.<sup>20</sup>; age:  $22.8 \pm 4.8$  years; height:  $169.9 \pm 3.5$  cm; body mass:  $68.5 \pm 8.5$  kg, average weekly training:  $14.3 \pm 2.8$  hours). Additionally, participants limb dominance was recorded (self-reported). All principles of the Declaration of Helsinki were followed, written informed consent was obtained prior to participation, and approval was granted by the University of Maia Ethics Committee (project 97/2022). The study protocol was registered within ClinicalTrials.gov, under the code: NCT06913036. For inclusion, participants were required to be male, healthy, absent from current or recent injury, aged between 18 and 35 years old, and have at least one year of experience in competitive road cycling. This research was carried out fully in accordance with the ethical standards of the *International Journal of Exercise Science*.<sup>21</sup> Sample size was calculated a-priori using G\*Power (version 3.1.9.7), for the paired-samples t-test (2-tailed) assuming an effect size of  $d = 0.9$ <sup>16</sup>, a statistical power of 80%, and an alfa level of 0.05.

### Protocol

This study collected measures of LFF at baseline (30 minutes before), 30 minutes post-exercise, and at the 24-hour mark following the exercise protocol. LFF was measured prior to warming up (to avoid any potentiation or fatigue effect), which consisted of 15 minutes of stationary cycling, starting at 60% of HRmax, with the intensity gradually increasing to 70% (5 minutes at 60%, 65%, and 70%), followed by a 6-second sprint<sup>22</sup>. To limit external influences, athletes were instructed to refrain from physical activity during the data collection period and maintain their nutritional and sleep routines. The synthesis of the experimental procedures is displayed in figure 1.



**Figure 1.** Study procedures and timeline. Notes: 30' TT - 30 minute time-trial; 4' TT - 4 minute time-trial.

### Low-frequency Fatigue Assessment.

LFF was assessed using the Myocene device (Myocene, Liège, Belgium)<sup>23</sup>. Participants were seated on the device with their leg positioned against the "Myo-sensor", a force sensor that captured evoked forces at a 4 kHz sampling rate. Electrical muscle stimulation was delivered using biphasic square waves with a 400  $\mu$ s pulse width, applied through three electrodes (MyoPro-1-electrodes, Myocene, Liège, Belgium). A cathode ( $5 \times 10$  cm) was placed horizontally across the proximal quadriceps femoris, while two anodes ( $5 \times 5$  cm each) were positioned over the vastus lateralis and vastus medialis. The stimulation protocol comprised 16 sets, each including a single pulse, a train of five stimuli at low frequency (20 Hz), and a train of 18 stimuli at high frequency (120

Hz), with one second separating each stimulus within a set, and five seconds of rest between sets. Stimulation intensity increased progressively by 1 mA per set, starting at 25 mA and reaching up to 40 mA, standardized across participants without individual adjustments. Participants were fully relaxed during all measurements and exited the device between test and retest sessions, being repositioned with the same 90° knee angle. The stimulation followed the standardized Myocene protocol, lasting approximately two minutes, and the device's algorithm computed the LFF score based on the ratio of forces evoked at low and high frequencies, using the median value of these ratios<sup>14,16</sup>. Higher LFF scores indicate greater muscular compliance (i.e., preserved muscular status), whereas smaller scores indicate more pronounced fatigue.

#### Time Trial Performance.

Following the warm-up, participants performed a 30-minute maximal self-paced time trial (30'TT) on Day 1 and a 4-minute maximal self-paced time trial (4'TT) on Day 2, with a 24-hour washout interval between sessions. Both efforts were completed on a stationary trainer (Tacx Neo 2T Smart), which was calibrated prior to each use. Heart rate (beats per minute) and power output (watts) were recorded using a Polar H10 chest band (Polar Electro Oy) and Favero Assioma Duo pedals<sup>24</sup> (calibrated prior to each session), respectively.

#### Perceptual Assessments.

##### *Perceived Muscle Soreness.*

Muscle soreness was evaluated using a 7-point Likert scale, where 0 represented "no soreness" and 6 indicated "severe soreness that restricts movement".<sup>25</sup> Participants completed this assessment at each time point. Instructions on how to use the scale were provided to participants prior to the baseline assessment.

##### *Perceived Fatigue.*

Participants' perceived fatigue was measured using a 10-point Rating of Fatigue scale, which includes descriptors ranging from "no fatigue at all" to "complete exhaustion – nothing remaining", in its translated version.<sup>26</sup> Instructions on how to use the scale were provided to each participant prior to the baseline assessment.

#### *Statistical Analysis*

All statistical analyses were conducted using R in RStudio (version 2025.05.0+496) for MacOS. Initially, the distribution of the data was visually inspected and complemented with the Shapiro-Wilk test, while homogeneity of variances was tested using Levene's test. LFF scores were analyzed using a linear mixed-effects model (lme4 and lmerTester packages) to examine the effects of protocol duration (30'TT vs 4'TT), time point (pre, post 30 minutes, and post 24 hours), and limb dominance (dominant vs. non-dominant). The model included protocol, time, and limb as fixed effects, along with their interactions. A random intercept was included for each participant ID to account for within-subject variability, and an unstructured covariance matrix was used. Fixed effects were tested using an ANOVA with Satterthwaite's approximation for degrees of freedom. Pairwise post-hoc comparisons were conducted using estimated marginal

means (emmeans package) with Holm-Bonferroni correction, and statistical significance was set at  $p < 0.05$ . Additionally, time trial performances were compared using a paired-samples t-test. Effect sizes were computed for both main effects and post-hoc comparisons, with partial eta-squared ( $\eta^2$ ) used for the mixed model analysis and Cohen's  $d$  ( $d$ ) for pairwise comparisons (effectsize package). The interpretation of  $\eta^2$  was as follows:  $\geq 0.01$  small,  $\geq 0.06$  medium, and  $\geq 0.14$  large.<sup>27</sup> Cohen's  $d$  was interpreted as  $\geq 0.2$  small,  $\geq 0.5$  medium, and  $\geq 0.8$  large.<sup>28</sup> Model assumptions (normality of residuals, homogeneity of variance, collinearity, linearity) were assessed through visual inspection of residual plots and quantile-quantile plots (performance and see packages).

The associations between perceived fatigue and muscle soreness with LFF scores across time points were assessed using repeated measures correlation (rmcorr package). Values were interpreted as follows: trivial ( $rm_{corr} < 0.1$ ), weak ( $0.1 \leq rm_{corr} < 0.3$ ), moderate ( $0.3 \leq rm_{corr} < 0.5$ ), large ( $0.5 \leq rm_{corr} < 0.7$ ), very large ( $0.7 \leq rm_{corr} < 0.9$ ), and nearly perfect ( $rm_{corr} \geq 0.9$ ).<sup>29</sup>

## Results

Statistically significant differences were observed for time trial power output (30'TT:  $247 \pm 32.5$  vs. 4'TT:  $345 \pm 36.7$ ;  $d = 3.79$ ;  $p < 0.001$ ), but not for heart rate (30'TT:  $169 \pm 12.9$  vs. 4'TT:  $171 \pm 9.5$ ;  $d = 0.24$ ;  $p = 0.309$ ).

The linear mixed-effects model revealed a significant main effect of time on LFF scores ( $F(218.12) = 126.47$ ;  $\eta^2 = 0.54$ ;  $p < 0.001$ ), indicating changes in muscle function across time points. Specifically, post-hoc pairwise comparisons highlighted significant differences between pre-exercise and 30 minutes post-exercise moments ( $d = 1.87$ ,  $p < 0.001$ ), as well as between 30 minutes post-exercise and the 24 hours post-exercise ( $d = -1.85$ ;  $p < 0.001$ ) (Fig. 2 A and B). Importantly, no significant differences were detected between baseline LFF values measured before each protocol (30'TT:  $77.8 \pm 5.5$  vs 4'TT:  $81.0 \pm 3.0$ ;  $d = 0.10$ ;  $p = 1.00$ ), suggesting that pre-exercise levels were comparable despite the fixed testing order.

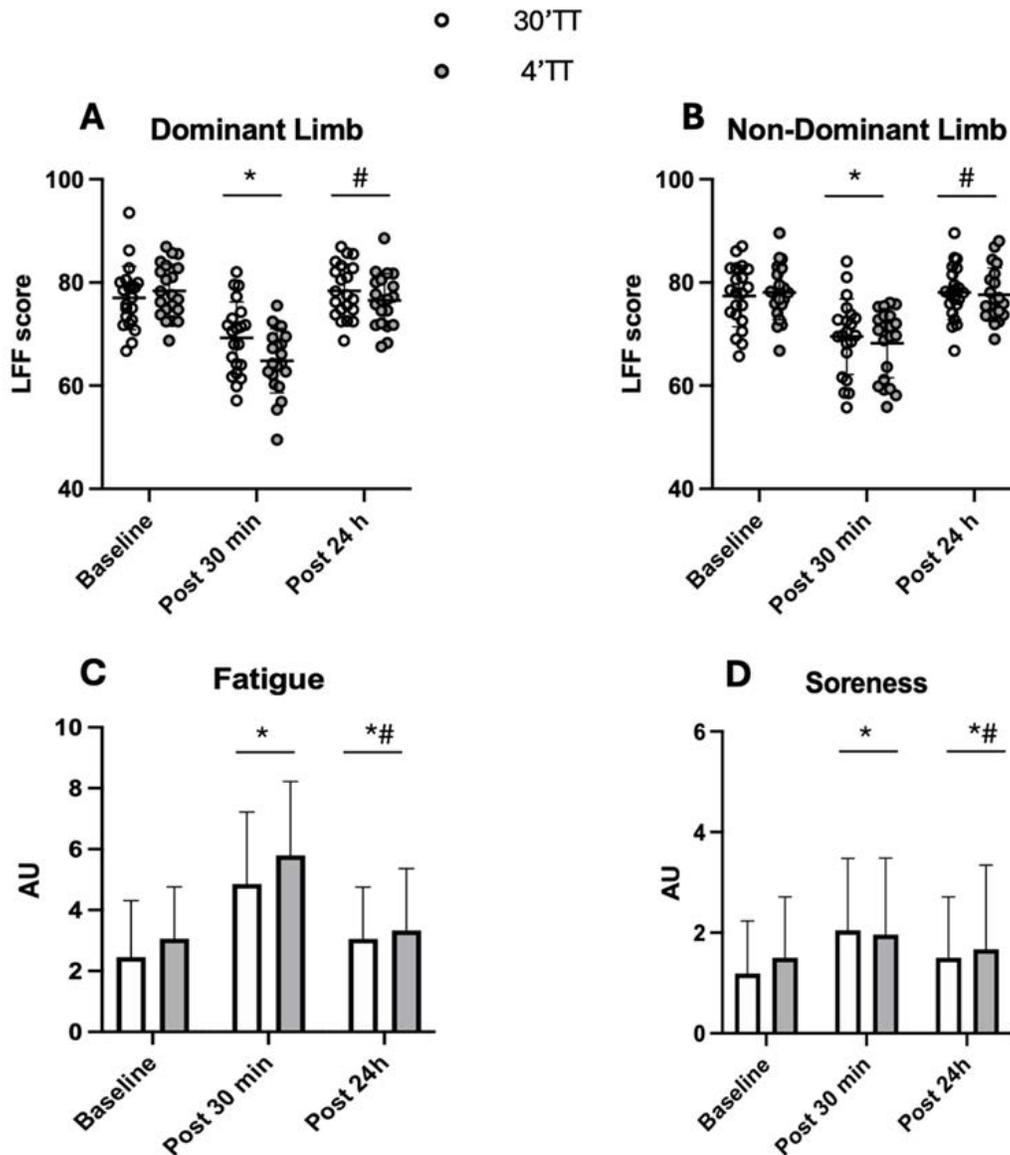
A significant time  $\times$  protocol interaction was also observed ( $F(218.12) = 3.80$ ;  $\eta^2 = 0.03$ ;  $p = 0.024$ ), highlighting that the time course of muscle function differed between the 30'TT and 4'TT protocols. Specifically, a significant more pronounced fatigue (i.e., lower score) was observed at the post 4'TT, compared with the post 30'TT ( $d = 0.39$ ;  $p = 0.032$ ). Additionally, significant differences were identified between pre- and post-exercise on the 30'TT ( $d = 1.06$ ;  $p < 0.001$ ), as well as in the 4'TT ( $d = 1.59$ ;  $p < 0.001$ ). Furthermore, differences between post-exercise values and the 24-hour time point were identified for both the 30'TT ( $d = 1.2$ ;  $p < 0.001$ ) and 4'TT ( $d = 1.42$ ;  $p < 0.001$ ).

Conversely, no significant main effects were found for protocol ( $F(218.12) = 2.82$ ;  $\eta^2 = 0.01$ ;  $p = 0.094$ ), limb dominance ( $F(218.03) = 1.75$ ;  $\eta^2 = 0.008$ ;  $p = 0.187$ ), time  $\times$  limb dominance ( $F(218.03) = 0.92$ ;  $\eta^2 = 0.008$ ;  $p = 0.400$ ), protocol  $\times$  limb dominance ( $F(218.03) = 1.29$ ;  $\eta^2 = 0.006$ ;  $p = 0.256$ ), or time  $\times$  protocol  $\times$  limb dominance ( $F(218.03) = 0.92$ ;  $\eta^2 = 0.008$ ;  $p = 0.398$ ).

While perceived fatigue (Fig. 2C) did not differ significantly between conditions at any timepoint, significant effects of time were detected between baseline and 30 minutes post-exercise ( $d = 1.69$ ;

$p < 0.001$ ), baseline and 24 hours post-exercise ( $d = 0.48$ ;  $p = 0.008$ ), and 30 minutes post-exercise and 24 hours post-exercise ( $d = 1.23$ ;  $p < 0.001$ ).

Similarly, for perceived soreness (Fig. 2D), no differences were detected between groups at each time point; however, significant effects of time were detected. Specifically, differences between baseline and 30 minutes post-exercise ( $d = 1.02$ ;  $p < 0.001$ ), baseline and 24 hours post-exercise ( $d = 0.51$ ;  $p = 0.004$ ), and 30 minutes post-exercise and 24 hours post-exercise ( $d = 0.55$ ;  $p = 0.002$ ).



**Figure 2.** A - Low-frequency fatigue scores across time points for the dominant limb; B - Low-frequency fatigue scores across time points for the non-dominant limb; C - Perceived fatigue scores across time points; D - Perceived soreness scores across time points. Notes: \* indicates statistical significance ( $p < 0.05$ ) between baseline and 30 min post-exercise; # indicates statistical significance between 30 min post-exercise and 24 hours post-exercise. Given the ordinal nature of the scales, the results are presented in bar charts. 30'TT - 30 minute time trial; 4'TT - 4 minute time trial; AU - arbitrary units.

The repeated measures correlation (Table 1) between LFF values and perceived fatigue was significant ( $rm_{corr} = -0.50$ ;  $p < 0.001$ ) indicating a large negative association. This suggests that as LFF levels decreased, perceived fatigue increased. For muscle soreness, a weak but significant repeated measures correlation was found between LFF scores and muscle soreness ( $rm_{corr} = -0.28$ ;  $p < 0.001$ ), suggesting a negative, yet weak association, where higher LFF values were associated with lower muscle soreness. Furthermore, perceived fatigue appeared to be associated with perceived muscle soreness, as a large association was identified ( $rm_{corr} = -0.56$ ;  $p < 0.001$ ) (Table 1).

**Table 1.** Repeated measures correlation between low-frequency fatigue (LFF), perceived fatigue, and muscle soreness.

	LFF	Fatigue
<b>Soreness</b>	-0.28* [-0.40 to -0.15] (weak)	0.56* [0.47 to 0.65] (large)
<b>Fatigue</b>	-0.50* [-0.59 to -0.39] (large)	

\*Denotes statistical significance ( $p < 0.05$ ). Values are  $rm_{corr}$  [95% confidence interval].

This study aimed to investigate potential differences in low-frequency fatigue (LFF) responses following two different cycling efforts—a maximal self-paced 30'TT and a 4'TT—in high-level road cyclists. The results show that LFF is responsive to fatigue induced by both efforts; however, the 4'TT elicited significantly lower scores of LFF (i.e., higher fatigue) compared to the 30'TT, suggesting higher neuromuscular strain despite the shorter duration. Additionally, a strong association was observed between perceived fatigue and LFF scores.

LFF is regaining attention in the scientific literature as an indicator of peripheral fatigue.<sup>14,16</sup> Accordingly, this study characterized its response following cycling efforts of different durations. We hypothesize that the observed differences between the protocols are primarily attributable to differences in force and power output (mean difference of 98 watts).<sup>30</sup> Since LFF reflects impairments in the musculature, it might be particularly sensitive to peripheral mechanisms of fatigue.<sup>16</sup> Therefore, short maximal efforts, such as the 4'TT, likely generate greater absolute power output, faster metabolite accumulation, higher motor unit recruitment patterns, and a higher contribution of anaerobic system, which may explain the larger LFF response when compared to longer-lasting efforts, such as the 30'TT.<sup>31</sup> In contrast, longer-duration efforts, while fatiguing, may rely more heavily on pacing strategies and central regulation, leading to central fatigue, with a less pronounced peripheral decline.<sup>32</sup>

Furthermore, the reasoning behind the decrement in LFF scores can be attributed to the disproportionate effect of fatigue on low- versus high-frequency muscle stimulations.<sup>23</sup> Under fatigue, the force produced in response to low-frequency electrical stimulation tends to decline more than that produced at higher frequencies.<sup>15</sup> Thus, a smaller LFF score reflects a considerable drop in force production at low frequencies, while high-frequency responses remain relatively stable.<sup>15</sup> This pattern indicates that peripheral fatigue primarily impairs the muscle's ability to generate force at lower frequencies, which defines the LFF metric.

Another relevant finding from our study is that LFF values had returned to near-baseline levels 24 hours after both exercise conditions. This could indicate that the protocols used did not induce sufficient fatigue to cause prolonged impairments, or, alternatively, that LFF may not be sensitive to fatigue processes that persist beyond the short term. It is plausible that longer efforts (e.g., 3-hour protocols) might induce more pronounced or longer-lasting LFF compared to shorter, high-intensity protocols, which may warrant further investigation. In addition to exercise duration, the mode of muscle contraction can also influence LFF, with evidence suggesting that eccentric actions might cause greater impairments.<sup>16</sup> Future research should investigate whether prolonged submaximal exercise elicits a higher degree of LFF and whether these measures, alongside different modes of contraction, are more responsive in such contexts compared with high-intensity actions.

Interestingly, a large association was found between LFF and perceived fatigue, which may indicate that greater neuromuscular impairments were consistently associated with higher subjective ratings of fatigue within participants. In contrast, a weaker association was observed between LFF and muscle soreness. The association between perceived fatigue and LFF in our study is similar to the findings of Tito et al.<sup>14</sup>, in soccer players, where moderate correlations ( $r = -0.43$ ) were reported. However, in their study, a large association was found between LFF and muscle soreness, which contrasts with our results. These discrepancies could be due to several factors, such as differences in the participants' familiarity with the soreness scale or, most likely, the mode of exercise employed. Soccer matches commonly lead to a greater degree of muscle soreness compared to the cycling efforts used in our study, due to the frequent actions such as accelerating, decelerating, sprinting, and jumping.<sup>33</sup> In contrast, our study involved steady-state cycling, which may have induced a smaller degree of soreness, especially among highly trained individuals familiarized with such efforts.

Taken together, these findings seem to support the utility of LFF as a sensitive marker of peripheral fatigue in cycling. Its responsiveness to different exercise durations and its strong association with perceived fatigue reinforce its potential value in both research and "on field" fatigue monitoring contexts. Nonetheless, this study has some limitations that must be acknowledged. Firstly, the order of exercise protocols was not randomized due to logistical constraints, as the study was conducted during a pre-planned structured training with a fixed schedule. This design could have introduced potential sources of bias in the results, such as cumulative fatigue, possible learning effects, increased pacing familiarity, or motivational adaptations across sessions. For example, participants may have performed the second protocol with greater efficiency or altered effort regulation due to familiarity with testing procedures, which could have influenced the magnitude of neuromuscular fatigue observed. Although LFF values had returned to baseline within 24 hours following each protocol, suggesting that fatigue responses were largely transient and carryover effects minimal, the fixed order may have inflated the perceived difference between protocols. Therefore, caution is warranted when interpreting between-protocol comparisons. Future studies should employ randomized counterbalanced designs to disentangle the effects of protocol characteristics from potential order-related influences, ensuring that observed differences in LFF truly reflect exercise-specific fatigue responses. Secondly, our results may not be generalizable to all populations, as this study only included male, highly trained participants. Specifically, different patterns may be observed with amateur or female cyclists. Also, although

the a priori sample size calculation was based on the detection of relatively large effects, smaller but potentially relevant effects may not have been fully captured and should be interpreted with caution. Finally, while LFF seems to be a valid measure of peripheral fatigue, no additional functional or biochemical assessments were conducted, which may limit the ability to draw broader conclusions.

This study provides new insights into the sensitivity of LFF following two different cycling efforts in high-level road cyclists. The results suggest that both short, high-intensity (4'TT) and longer, sustained (30'TT) efforts performed at maximal intensity induce LFF, with a slightly more pronounced response observed following the 4-minute protocol. Although more research is warranted, our results seem to suggest that LFF can be sensitive to immediate changes (i.e., post-exercise) in cycling and used as a tool to monitor peripheral fatigue. Additionally, our study highlights a strong association between perceived fatigue and LFF scores, further suggesting that LFF could serve as an objective marker of physical fatigue.

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*Conflicts of Interest:* The authors declare the absence of conflicting interests.

*Protocol and Registration:* ClinicalTrials.gov under the code NCT06913036.

*Ethics Approval:* This study was approved by the University of Maia Ethics Committee (project 97/2022).

*Data availability:* The datasets from the current research are available from the corresponding author on reasonable request.

*Code availability:* The script used in this study is available from the corresponding author on reasonable request.

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