## **EffEcts of BEEt JuicE on PErformancE in comPEtitivE swimmErs**

**EfEctos dEl Zumo dE rEmolacha soBrE El rEndimiEnto En nadadorEs comPEtitivos**

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Effects of Beet Juice in Swimmers

**Short title:**

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#### **Abstract**

This study analysed the effect of beetroot juice supplementation (BRJ) in an intermittent swimming session with high glycolytic demands on performance, biomechanical variables (speed, stroke length, cycle frequency, stroke index), blood lactate concentrations (BLa) and rating of perceived exertion (RPE). This study design was randomized, double-blind, cross-over and placebo-controlled. Ten competitive adolescent swimmers (six females and four males, range of age from 13 to 16 years) executing a swimming test consisting of an 8 x 50 m swimming protocol after taking BRJ (~ 12.8 mmol of nitrate) or a placebo (PLA). Biomechanics parameters, RPE after each set, and BLa pre and post-exercise were assessed. BRJ enhanced the time in the swimming test  $(p = .004)$ with a best medium time (*p =* .004) and time in the slowest set (*p =* .041). No differences were reported between BRJ and PLA for biomechanical parameters, BLa or RPE. Based on the results of the present study, it is concluded that BRJ could enhance swimming performance and the relationship between work and RPE, without any effect on the biomechanics parameters. Therefore, the intake of BRJ could be beneficial for swimmers along the high-intensity intermittent sessions.

*Keywords:* Aquatic sport, swimmer, ergogenic aid, nitrate, nitrite, supplement.

# **Resumen**

Este estudio analizó el efecto de la suplementación con zumo de remolacha (BRJ) en una sesión de natación intermitente con altas demandas glucolíticas sobre el rendimiento, variables biomecánicas (velocidad, longitud de brazada, frecuencia del ciclo, índice de brazada), concentraciones de lactato en sangre (BLa) y índice de esfuerzo percibido (RPE). El diseño de este estudio fue aleatorio, doble ciego, cruzado y controlado con placebo. Diez nadadores adolescentes competitivos (seis mujeres y cuatro hombres, rango de edad de 13 a 16 años) ejecutaron una prueba de natación consistente en un protocolo de nado de 8 x 50 m después de tomar BRJ (~ 12.8 mmol de nitrato) o un placebo (PLA). Se evaluaron los parámetros biomecánicos, RPE después de cada serie y BLa antes y después del ejercicio. BRJ mejoró el tiempo en la prueba de natación (*p =* .004) con un mejor tiempo medio (*p =* .004) y tiempo en la serie más lenta (*p =* .041). No se informaron diferencias entre BRJ y PLA para los parámetros biomecánicos, BLa o RPE. En base a los resultados del presente estudio, se concluye que el BRJ puede mejorar el rendimiento en natación y la relación entre trabajo y RPE, sin tener ningún efecto sobre los parámetros biomecánicos. Por tanto, la ingesta de BRJ podría ser beneficiosa para los nadadores durante las sesiones intermitentes de alta intensidad.

*Palabras clave:* Deporte acuático, nadador, ayuda ergogénica, nitrato, nitrito, suplemento.



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## **Introduction**

Swimming is one of the Olympic sports that has improved the most over the last years, with world records being broken regularly during the Olympic cycle. The biomechanical, energetic, and training methodology aspects are the main responsible for the evolution of this sport (Terzi et al., 2021). Parameters as stroke length (SL), cycle frequency (SF), stroke index (SI) and highest blood lactate (BLa) concentration in post-exercise condition are essential biomechanical and energetic factors for swimming performance (Marinho et al., 2020). In swimming season planning there are different training-intensity distribution models of periodization. Among the two most commonly used methodologies are traditional training (TT), characterized by high-volume, low-intensity training, and ultra-short race-pace training (USRPT) (Williamson et al., 2020). USRPT is a methodology used to increase a swimmer's performance through a large number of swimming sets at velocities similar to competitive velocities using short distances and short recovery periods. These two training methodologies are very different, but both methodologies have some similarities when comparing certain stages of their programming (Nugent et al., 2017). During various phases of TT, exercises resembling those in USRPT training are utilized to improve both oxidative and glycolytic capacity, to elicit and accumulate elevated BLa.

Increasing glycolytic capacity is essential for distances from 50 to 400 m. Various training tasks such as 4x50 m, 6x50 m, and 8x50 m, with several recovery periods are used to stimulate high BLa concentrations (Terzi et al., 2021) and to improve non oxidative capacity (Kabasakalis et al., 2019). These tasks promote ions of hydrogen (H+) accumulation, the development of fatigue, and a decrease in the intramuscular capacity of the muscle groups involved. When performing these tasks, the glycolytic capacity of the swimmer can be optimized. It can be checked by a reduction of BLa at a same swimming velocity, favoring the metabolic activity and the ability to produce force by the muscle groups involved, and thus increase their performance (Faghy et al., 2019). Also, the swimming velocity is dependent on the relationship between SF and SL. This relationship is affected by the energy cost of swimming (Cs) that increases when SF increases. SI is a swimming efficiency variable that at a certain speed, indicates that the swimmer who gets the greatest cycle distance per stroke also has the most efficient swimming technique. Also, higher levels of SI are correlated to low Cs (Lätt et al., 2010). Combining these metabolic and biomechanical adaptations using these tasks can result in higher performance for the swimmer.

In competitive swimming, differences of 1.6% distinguish a is conceivable between a gold medalist and a fourth- place in an Olympic Games (Mujika et al., 2002). In this context, 86.9% of a sample of national and international competitive level declared consuming at least one sport supplement with the main motivation for enhancing sport performance (Moreno et al., 2022). At the difference of caffeine supplements, sport drinks, or sport bars which are consumed by more than half of the competitive level swimmers, beetroot juice (BRJ) is not one of the sport supplements more consumed by swimmers (Moreno et al., 2022). However, it has been proposed as an ergogenic aid with a high level of scientific evidence (Maughan et al., 2018). BRJ is an enriched dietary source of inorganic nitrate (NO3-). Approximately 20% of NO3- dietary intake is reduced through the salivary glands to nitrite (NO2-) by commensal anaerobic bacteria on the dorsal surface of the tongue (Ahmed et al., 2017), whereas NO2- is partially reduced in the stomach into nitric oxide (NO). Thereby, NO3- supplementation increases systemic NO and NO2-, which could be further reduced into NO in hypoxia and acid conditions at the muscular level (Bailey et al., 2012), where it exists a reservoir of NO2- (Nyakayiru et al., 2020). Elevation of NO after NO3– supplements as BRJ increases capacity for muscular work and/or improves muscle contractile efficiency (San Juan et al., 2020). Nevertheless, the ergogenic properties of sport supplements depend on the mechanical and energy demands of each type of sports modality with possible ergogenic effects for some types of efforts and no effect for other types (Domínguez et al., 2018; Larrosa et al., 2024). Today, only four studies have analyzed the effect of BRJ on competitive swimmers (Esen et al., 2019; Lowings et al., 2017; Moreno et al., 2023; Pospieszna et al., 2016), but no studies have analyzed the effect of BRJ on biochemical parameters during swimming that influence in swimming performance as swimming velocity, SL, SF or SI. Therefore, the aim of this study was to analyze the effect of BRJ compared to placebo on the performance, biomechanics variables (swimming velocity, SL, SF and SI), BLa, and RPE during a session with high glycolytic demands (8x50 m) in competitive swimmers.

## **Methods**

#### **Design and Procedure**

The study design was randomized, double-blind, cross-over, and placebo-controlled. Participants completed two experimental visits to the swimming pool. Randomly 50% of the participants (*n* = 5) were assigned to ingest 140 ml of BRJ enriched in NO3- (∼12.8 mmol of NO3-) whereas the rest of the participants (*n* = 5) ingested as placebo (PLAC) 140 ml of BRJ depleted in NO3- (.08 mmol of NO3-) (Research Randomizer, www.randomizer.org). In the second session, participants who ingested BRJ enriched in NO3- during the first experimental session were supplemented with PLAC whereas participants who took PLAC in the first sessions ingested BRJ enriched in NO3-. Experimental visits were separated for 48 hours. Experimental sessions were performed in the evening at the same time of the day (± .25 hours) to avoid a possible interaction of the effects of BRJ based on the time of day. During each experimental session, after a standardized warm-up, participants executed 8 repetitions of 50 m at maximal speed with a passive recovery of two minutes. After each repetition, RPE was administered. In addition, it was assessed blood lactate concentrations (BLa) before starting the warm-up (BLaPRE) and two minutes after the last 50 m repetition (BLaPOST).

#### **Participants**

The sample of this study was formed by 10 competitive adolescent swimmers (6 females and 4 males, range of age from 13 to 16 years). All the participants won a gold or silver medal in the regional competition the week before the start of the study. All the participants were normotensive, physically active, and summed an experience of systematically swimming training (> 5 sessions training per week) higher than three years. All the participants were familiarized with the test used because it was used frequently during the training session. Before the start of the study, participants and their parents were meeting with a researcher who informed about the characteristic of the study. After being fully informed of the experimental protocols, all the parents signed an informed consent for participating in this study. This study was in accordance with the Declaration of Helsinki, and it was approved by the Ethics Commission of the Polytechnic Institute of Setúbal (code: PI26/2022).

For the characterization of the sample, an anthropometric evaluation was assessed. Height was assessed using a stadiometer BSM170B Stadiometer (Inbody Co. Ltd, Cerritos, CA, USA) with participants barefoot and ensuring the frankfort plane. In addition, it was used a bioimpedance using an Inbody 270 body composition analyzer (Inbody Co. Ltd, Cerritos, CA, USA) for estimating body mass muscle mass, % body fat, and body mass index (BMI).

### **Supplementation and Dietary Control**

One hundred fifty minutes before the warm-up participants arrived at the swimming pool and they ingested 140 mL of a BRJ supplement enriched in NO3- (~ 12.8 mmol NO3-) (Beet IT; James White Drinks Ltd, Ipswich, UK) or a PLAC attending to previous instructions (Domínguez et al., 2018). This timing was adjusted based on the plasma NO2- peaks (2-4 hours post-ingestion) after an intake of 140 ml of a BRJ supplement (Wylie et al., 2013). During the 24 hours preceding experimental sessions was standardized a diet to ensure a similar distribution of protein (10%), carbohydrates (60%) and lipids (30%). Also, it was restricted during this time NO3— rich foods (beetroot, turnip, celery, arugula, lettuce, spinach, leak, cabbage, endives, parsley) and caffeine dietary sources (energy drinks, coffee, mate, tea soft drinks, tea, cola drinks, chocolate drinks and chocolate). In addition, during 24 hours prior to each experimental session, participants were refrained for brushing their teeth or using a mouthwash, chewing gum, or eating any sweets that could contain bactericidal substances such as xylitol or chlorhexidine.

#### **Swimming Test**

The swimming test consisted of eight repetitions of 50 m freestyle performed at maximal speed with two minutes of passive recovery between repetitions. The test was performed in a swimming pool of 25 m. Before the test, participants performed a standardized warm-up consisting of four repetitions of 200 m freestyle with 45 seconds of rest between repetitions and four repetitions of 12.5 m at maximum intensity with 45 seconds of recovery between each repetition. For each repetition, it was measured the total time, SF, SL, and SI by two experienced researchers. Swimming velocity was obtained from the mean value of each lap (from 5 m to 20 m). SF was registered from the mean of three consecutive stroke cycles in the middle of the pool of each lap, using a chrono-frequency meter (Golfinho Sports MC 815, Aveiro, Portugal). Later, SF was converted to International System units (Hz). SL was calculated from the ratio of elapsed time to the SF. SI resulted from swimming velocity times SL (Marinho et al., 2020). All biomechanics values resulted from the mean of the two experienced researchers that were blinded to the swimmers. To confirm the accuracy of all measurements intraclass correlation coefficient was determined for the time, SF, SL, and SI.

#### **Rating of Perceived Exertion**

At the end of each 50 m repetition, a 10 point Borg scale was administered to record the ratings of perceived exertion (RPE).

#### **Blood Lactate Concentrations**

Previous to the warm-up and two minutes after the end of the swimming test, capillary blood samples (5 µL) were obtained from the pad of the left thumb for assessing BLa using a Lactate ProTM 2 LT-1710 blood analyzer (Arkray Factory Inc., KDK Corporation, Shiga, Japan).

### **Statistical Treatment**

The normality distribution of the data was confirmed using a Shapiro-Wilk test whereas homoscedasticity was confirmed by the Levene test. Results are presented as mean (*M*) ± standard deviation (*SD*) values. An ANOVA-RM for the factors time (repetitions), supplementation (BRJ vs PLAC), and time·supplementation were performed for time, RPE, speed, SF, SL, and SI. In addition, an ANOVA-RM for the factors time (pre vs. post) supplementation and time·supplementation was performed for BLa. In addition, effect size (*ES*) was calculated using partial eta squared (*η\_p^2*), with .25 considered as small; .26 - .63 considered as medium; and > .63 considered as large. In addition, it was performed a paired t-test for detecting

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possible differences between BRJ and PLAC in speed, SF, SL, and SI registered in the best and the worst repetition for each participant under the two experimental conditions. Also, in the pairwise comparisons, *ES* was calculated using Cohen's d considering lower than .2 as trivial; .2 - .5 as small; .5 - .8 as moderate; and .8 as large. Statistical differences were set up *p* < .05. All the analyses were performed in the SPSS software (SPSS Inc., V.21, Chicago, IL, USA).

#### **Results**

**Table 1**

Characteristics of the participants are presented in Table 1.



*Note:* Data expressed mean ± standard deviation (*SD*).

The ANOVA-RM reported statistical differences and a large ES for supplementation with a lower time for BRJ compared to PLAC (*F* = 5.094; *p =* .004; *η\_p^2* = .626), but no statistical differences were reported for time (*F* = 8.868; *p =* .055; *η\_p^2* = .287) nor time·supplementation (*F* = .738; *p =* .512; *η\_p^2* = .076) (see Figure 1).



**Figure 1** *Time (s) of each 50 m repetition in the swimming test*

*Note:* Data presented as *M* ± *SD*.

Regarding the biomechanics variables, it was not reported differences in the speed for the factors time (*F* = 3.287; *p =* .065; *η\_p^2* = .267), supplementation (*F* = .406; *p =* .540; *η\_p^2* = .043) nor time·supplementation (*F* = 1.379; *p =* .274; *η\_p^2* = .133) (see Figure 2a). For SF was reported differences for time (*F* = 4.353; *p =* .014; *η\_p^2* = .326), but not for supplementation (*F* = .666; *p =* .436; *η\_p^2* = .666) nor time·supplementation (*F* = .484; *p =* .709; *η\_p^2* = .051) (Figure 2b) whereas for SL not differences were found nor time (*F* = .801; *p =* .446; *η\_p^2* = .082), supplementation (*F* = 2.056; *p =* .185; *η\_p^2* = .186) nor time·supplementation (*F* = .324; *p =* .115; *η\_p^2* = 1.670) (see Figure 2c). The analysis of the SI didn´t report statistical differences for time (*F* = 3.609; *p =* .054; *η\_p^2* = .254), supplementation (*F* = 1.128; *p =* .316; *η\_p^2* = .111) nor time·supplementation (*F* = 1.549; *p =* .243; *η\_p^2* = .147), but in the pairwise comparison was reflexed an enhanced SI for BRJ compared to PLAC only in the fastest 50 m (*BRJ*: 2.71 ± 0.48 *vs. PLAC*: 2.53 ± 0.47; *t* = -2.447; *p =* .037; *d* = .40) (see Figure 2d).

It was detected a progressively increase in RPE (*F* = 12.707; *p* < .001; *η\_p^2* = .585), but no differences for supplementation (BRJ: 7.44 ± 0.31 vs. PLAC: 7.75 ± 0.25; *F* = .001; *p =* .975; *η\_p^2* < .001) nor time·supplementation (*F* = .552; *p* = .573; *η\_p^2* = .058). Similar results were found in BLa with differences based on time (*F* = 275.945; *p* < .001; *η\_p^2* = .968), but not for supplementation (BRJ: 16.66 ± 2.50 vs. PLAC: 16.55 ± 2.81 mmol; *F* = .119; *p* = .739; *η\_p^2* = .013) nor time·supplementation (*F* = .026; *p =* .875; *η\_p^2* = .003).

*Speed (m/s) of each 50 m repetition, fastest, and lowest time in the swimming test* Speed (m/s)  $P LAC$ **BRJ** 1,60 1,40 1,20 1,00  $50m-1$  $50m-2$  $50m-3$  $50m-4$  $50m-5$  $50m-6$  $50m-7$  $50m-8$ 50m-Medium 50m-Fastest 50m-LOWEST

**Figure 2a**

*Note:* Data presented as *M* ± *SD*.

**Figure 2b** *Stroke frequency (Hz) of each 50 m repetition, fastest, and lowest time in the swimming test*



*Note:* Data presented as *M* ± *SD*.



**Figure 2c**

*Note:* Data presented as *M* ± *SD*.





*Note:* Data presented as  $M \pm SD$ . \*: statistical differences ( $p < 0.05$ ) between BRJ and PLAC.

### **Discussion**

This study analyzed the effects of BRJ on the performance in an 8x50 m swimming test, a task commonly used for training glycolytic capacity in swimmers. The main results of this study were the next: i) exists a significant effect on SF, but there isn´t affectation of time and the rest of biomechanics variables analyzed along the intermittent swimming protocol; ii) BRJ resulted in a positive effect for factor supplementation in the 8x50 m swimming test; iii) RPE progressively increased during the swimming test without any effect of BRJ; iv) BRJ do not affect to BLa after a swimming protocol for enhancing glycolytic capacity in swimmers.

The analysis of BLa<sub>poST</sub> concentrations (16.61 ± 0.75 mmol) shows an important contribution of glycolytic metabolism of the swimming test used which is suitable for stimulating the glycolytic capacity of swimmers during training. In fact, comparing with other studies with a same population the  $BL_{post}$  in this research is higher in comparison with other studies using the same swimming test (from  $10.5 \pm 2.9$  in female to  $14.2 \pm 2.5$  mmol in males to  $15.3 \pm 3.1$  mmol in females) (Kabasakalis et al., 2020), a 4x50 m protocol (from 14.1 ± 3.1mmol in males to 15.3 ± 3.1 mmol in females) (Terzi et al., 2021), a 2x4x50 m or 8x50 m (~ 13 mmol) (Kabasakalis et al., 2019) or 20x25 m protocols (Williamson et al., 2020). The high  $BLA<sub>post</sub>$  could establish an affectation of muscle metabolic activity and force production during the propulsive phase of the stroke (Faghy et al., 2019). These fatigue mechanisms could establish a change in the swimming pattern with a progressive diminution of SF for the maintenance of Cs (Lätt et al., 2010). Therefore, it is possible that swimmers could benefit more meters the underwater wave, since under water hydrodynamic forces are less than on the surface (Born et al., 2022). This hydrodynamic advantage could explain a strategy for maintaining speed and performance along the high intensity intermittent swimming test used in this study in a very highly trained sample.

The main novelty of this study is that BRJ presents a statistical effect along the 8x50 m protocol. In the analysis of BLa, it was not detected any effect of BRJ on glycolytic capacity because there is not any difference in the comparison with PLA. In the laboratory has been registered a decreased oxidative energy cost in swimming after BRJ (Pinna et al., 2014), in agreement with the results reported in other types of exercises (i.e., knee extension) (Bailey et al., 2010). An in vitro study has reported an improved of the intramolecular mechanisms involved during the muscle contraction in the muscle II type fiber (Hernández et al., 2012), which are the more sensitive to fatigue. Therefore, BRJ could acts as ergogenic in a progressively reduction of the fatigue which is originated along the 8x50 m protocol. Thus, different meta-analyses have reported an enhanced economy after BRJ (Pawlak-Chaouch et al., 2016) and time-to-exhaustion tests (TTE), but not in time trial tests (TT) (Van De Walle & Vukovich, 2018).

Specific studies in swimming reflect discrepancies (Esen et al., 2019; Lowings et al., 2017; Moreno et al., 2023; Pospieszna et al., 2016). Essen et al. (2019) failed to enhance swimming performance in a 100 m and a 200 m TT while Lowings et al. (2017) reported a trend to increase performance (*p =* .06) in the second half of a 168 m backstroke TT. By contrast using an intermittent procedure, Pospieszna et al. (2016) reported an enhancement in the four last sets of an intermittent test consisting in 6x50 m in female university swimmers after BRJ (10.2 mmol NO3-). More recently, another study has reported a trend to enhance the time in a 6x100 m protocol (Moreno et al., 2023), however, the dosage used (6.4 mmol NO3-) could be insufficient for detecting statistical differences. In this way, the ergogenic effect of BRJ is mediated by its capacity to increase NO levels versus baseline levels (Wylie et al., 2013). If NO is increased in a dosage relationship until 8.4 mmol NO3-, it is possible that the amount provided by Moreno et al. (2023) was insufficient for detecting statistical differences. Therefore, the results of the present studies and Pospieszna et al. (2016) suggest ergogenic properties of BRJ on high intensity intermittent protocol in swimming with dosages upper than 10 mmol NO3- in concordance with the results of meta-analysis focused on fatigue procedures (Van De Walle & Vukovich, 2018).

RPE is influenced by sensory feedback that includes the set of psychophysiological sensations integrating central and peripheral signals and is used as an indicator of different physiological parameters such as heart rate, BLa, or ventilatory response during exercise (Campos et al., 2021). Along the 8x50 m test, RPE was progressively increased in agreement with Barroso et al. (2015), who state that continuous exercise with constant intensity causes a linear increase in RPE, and that the volume of interval training affects the perception of effort and the swimmer's internal training load (Barroso et al., 2015). Similarly, with less distance but more repetitions than our study, Williamson et al. (2020) reported RPE increases after a 20x25 m protocol (18 ± 1.6). Nevertheless, however, the pattern of RPE along the swimming test is the normal answer to this type of exercise, the same RPE with a higher swimming performance after BRJ reflects ergogenic properties of BRJ on RPE. In this sense, the same RPE with a higher performance could be interpreted as a positive response because it enhances the relationship between work and RPE ratio (Jodra et al., 2020).

This study provides valuable insights into the potential benefits of BRJ on swimming performance and RPE during highintensity intermittent sessions in swimming, several limitations should be considered. Firstly, it has been stablished a correlation between the increasement of NO2- as response to the supplementation and the ergogenic effects (Jones et al., 2018). Therefore, the absence of the assessment of NO2- concentrations is a limitation of this study. This study has recruited to a mixed sample formed by male and female swimmers based on the underrepresentation of women in studies focused on the analysis of the effect of sport supplements (Smith et al., 2022). Nevertheless, it has been suggested a specific effect of this supplement based on sex (Wickham & Spriet, 2019). Attending to the small sample size of this study, a second limitation is that this study does not analysed the interaction of the supplementation and sex effect for detecting a possible response mediated by sex. Lastly, this is a pioneer study analysing the effect of BRJ in biomechanics parameters, however, it is not studied the physiological mechanisms that are involved with the instauration of fatigue. Therefore, future studies should include the response of NO2- to the supplementation, analyse a possible interaction to the supplementation mediated by sex and combine kinematic swimming parameters with physiological mechanisms involved in the instauration of fatigue.

### **Conclusions**

The results of this study reflect a positive effect of BRJ in an intermittent swimming protocol without affectation to biomechanics parameters or lactate production, but an increased work and RPE relationship. Also, responses to this protocol suggest that 8x50 m task could be effective for stimulating the glycolytic capacity of swimmers during training. In addition, it is possible that the increased performance during this task could favor adaptations to training and sport performance in highly trained swimmers.

### **Ethics Committee Statement**

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee: POLYTECHNIC INSTITUTE OF SETÚBAL (code: PI26/2022).

## **Conflict of Interest Statement**

The authors declare no conflict of interest associated with this article.

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## **Authors' Contribution**

Conceptualization L.L. & R.D.; Methodology L.L. & R.D.; Formal Analysis R.D.; Investigation L.L., A.J.S.-O. & R.D.; Resources L.L.; Data Curation L.L., A.J.S.-O. & R.D.; Writing – Original Draft L.L., A.J.S.-O. & R.D.; Writing – Review & Editing L.L., A.J.S.-O. & R.D.; Visualization L.L., A.J.S.-O. & R.D.; Funding Acquisition L.L. All authors have read and agreed to the published version of the manuscript.

## **Data Availability Statement**

The data that support the findings of this study are available on request from the corresponding author ([sanchezoliver@us.es\)](mailto:sanchezoliver@us.es).

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