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p-Synephrine, the main protoalkaloid of *Citrus aurantium*, raises fat oxidation during exercise in elite cyclists

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ABSTRACT

Purpose: The aim of this study was to investigate the acute effects of *p*-synephrine ingestion on substrate oxidation during exercise in elite cyclists. **Methods:** Fifteen elite cyclists volunteered to participate in a double blind, crossover, randomized and placebo-controlled experimental trial. During two different trials, participants either ingested a placebo (cellulose) or 3 mg/kg of *p*-synephrine. After 60 min for substances absorption, participants performed an incremental maximal cycle ergometer test until volitional fatigue (25 W/min). Breath-by-breath gas exchange data was continuously recorded during the entire test to estimate energy expenditure, carbohydrate oxidation, and fat oxidation rates by stoichiometric equations. Heart rate was continuously measured by using a heart rate monitor. **Results:** The ingestion of *p*-synephrine had no significant effects on energy expenditure (F=0.71, *P*=0.40) or heart rate (F=0.66, *P*=0.43) during exercise. However, there was a main effect of *p*-synephrine to increase the rate of fat oxidation over the placebo (F=5.1, *P*=0.04) and the rate of fat oxidation was higher with *p*-synephrine in the following loads: 45±2%, 51±3%, 62±3%, 67±4%, 79±5% and 85±5% of the maximum wattage obtained in the test (all *P*<0.05). The ingestion of *p*-synephrine did not modify the maximal rate of fat oxidation during the ramp test (mean value; 95%CI = 0.91; 0.79-1.03 vs 1.01; 0.91-1.11 g/min, respectively, *P*=0.06) nor the exercise intensity at which maximal fat oxidation was achieved (i.e., Fatmax= 49; 48-53 vs 50; 47-51% Wmax, *P*=0.52).

Conclusion: Acute *p*-synephrine ingestion moved the fat oxidation-exercise intensity curve upwards during an incremental cycling test without affecting Fatmax.

Keywords: nutrition; fat oxidation; *Citrus aurantium*; bitter orange; body weight loss; elite cyclists.

INTRODUCTION

Active contracting skeletal muscle primarily employs carbohydrate and fat as fuel during exercise in order to sustain energy demands while amino acid oxidation is minimal (Jeukendrup, 2003). The contribution of carbohydrate and fat to the oxidative production of energy can vary during exercise mainly depending on exercise intensity (Venables, Achten, & Jeukendrup, 2005). In absolute terms, the rate of carbohydrate utilization by the contracting muscle increases with exercise intensity. However, the relationship between fat oxidation rate and exercise intensity is parabolic because the highest contribution of fat oxidation to the working muscle occurs at moderate exercise intensity (Achten, Gleeson, & Jeukendrup, 2002). Other factors such as exercise time, training level, body adiposity, pre-exercise diet, and ambient temperature can also be modulated to modify the proportion of fat and carbohydrate oxidation during muscle contraction (Purdom, Kravitz, Dokladny, & Mermier, 2018).

Much attention has been paid to finding the most optimal conditions for fat oxidation during exercise, perhaps due to its potential to reduce body fat stores (Achten & Jeukendrup, 2004; Astorino & Schubert, 2018; Maunder, Plews, & Kilding, 2018). Particularly, it might be of important relevance for individuals that have the goal of reducing body fat in the long term but also for those who seek to spare glycogen during

sport competitions. One effective strategy of relevance is the employment of alkaloids to increase fat utilization during exercise. Alkaloids are naturally occurring organic compounds, generally containing basic nitrogen atoms, and obtained from seed plants, which possess potent pharmacologic effects. It is worth noting, however, that the alkaloids' efficacy to change substrate oxidation is inconsistent and it depends of the characteristics of the substance and its mechanism of effect (Kim & Park, 2016). *p*-synephrine (4-[1-hydroxy-2-(methylamino)ethyl]phenol), the principal alkaloid of bitter orange (*Citrus aurantium*), is commonly included in dietary supplements that claim to decrease body weight and body fat (Stohs, Preuss, & Shara, 2012). *p*-synephrine is a phytochemical that can potentially induce changes in substrate oxidation at rest and during exercise through stimulation of β-3 adrenoceptors (Ratamess et al., 2016; Ratamess et al., 2015) while the side effects derived from *p*-synephrine ingestion, such as increased resting heart rate and blood pressure, are generally minimal, especially in comparison to more active substances like caffeine (Bush et al., 2018).

We have previously demonstrated that ingesting at least 2 mg of *p*-synephrine per kg of body mass is necessary in order to increase the rate of fat oxidation, particularly at low-to-moderate exercise intensities (Gutierrez-Hellin & Del Coso, 2016, 2018a). During exercise, *p*-synephrine also reduces the rate of carbohydrate oxidation while energy expenditure is generally unchanged with this alkaloid (Gutierrez-Hellin & Del Coso, 2016; Gutiérrez-Hellín, Ruiz-Moreno, & Del Coso, 2019). Acute *p*-synephrine ingestion can also reduce perceived exertion during prolonged exercise (Haller, Duan, Jacob, & Benowitz, 2008), increase the number of repetitions during a squat resistance test (Ratamess et al., 2015) and increase fat utilization for 30 min after exercise (Ratamess et al., 2016). Furthermore, the combination of *p*-synephrine and caffeine does not induce a synergistic

effect on substrate oxidation during or after exercise beyond the effect found with the isolated ingestion of these two substances (Gutierrez-Hellin & Del Coso, 2018b; Jung et al., 2017; Ratamess et al., 2016).

All the aforementioned investigations have examined *p*-synephrine's effect on exercise fat oxidation by using study samples of healthy and recreationally active individuals. While aerobic training increases adipose tissue lipolysis and skeletal muscle fat oxidation (Nordby, Saltin, & Helge, 2006), there is no scientific evidence to determine whether *p*-synephrine might also increase fat oxidation in well aerobically-trained individuals. Thus, the objective of this research was to determine the substrate oxidation effects of *p*-synephrine during exercise in highly trained participants (i.e., elite cyclists). We hypothesised that acute *p*-synephrine ingestion would enhance fat oxidation during a graded exercise protocol, particularly at low-to-moderate exercise intensities, with no effect on energy expenditure.

METHODS

Participants. Fifteen young, elite road cyclists from a Spanish cycling team volunteered to participate in this investigation (Table 1). A pre-experimental sample size calculation indicated that we would need at least 13 participants to detect an increase in maximal fat oxidation of 0.17 g/min, with a power of 0.80 and two-tailed α level set at 0.05. Participants competed at national and international cycling events, possessed a semi-professional status and competed in a Continental cycling team determined by the *Union Cycliste Internationale* (UCI). They were considered elite road cyclists following the criteria for the classification of cyclists' level proposed by Jeukendrup, Craig, and Hawley (2000). All participants were non-smokers and had no previous history of serious

metabolic, musculoskeletal or cardiopulmonary diseases. None had suffered any musculoskeletal injuries in the six months prior to the study. Participants were encouraged to avoid medications, nutritional supplements, and sympathetic stimulants for the duration of the study. Compliance was examined with dietary questionnaires. One week prior to the study, participants were fully informed of the experimental procedures and the risks and discomforts associated with the protocol. Participants signed a consent form in order to participate in the investigation. The study was approved by the Camilo José Cela University Research Ethics Committee (reference: 04/2016) and the research protocols are in accordance with the latest version of the Declaration of Helsinki.

Experimental design. A double-blind, placebo-controlled cross-over experimental design was used in this investigation. Each participant completed two identical trials that were separated by 48 hours in order to allow recovery and substance elimination (Hengstmann & Aulepp, 1978). Through random assignment, participants ingested an unidentifiable capsule filled with either 3 mg of *p*-synephrine per kg of body mass (99% purity; Synephrine HCL, Nutrition Power, Spain) or 100% cellulose (Guinama, Spain) as the placebo. The use of 3 mg/kg of *p*-synephrine was based upon previous publications in active and healthy individuals (Gutierrez-Hellin & Del Coso, 2016, 2018b). The capsule was administered with 150 mL of tap water 60 min before the onset of the experimental trials.

An alphanumeric code was assigned to each trial by an individual independent to the study. Investigators and participants were not aware of the assignment or substances. Ambient temperature and humidity were recorded at the beginning and at the end of each trial using a digital temperature and humidity monitor (OH1001, OH Haus, Spain). The

average ambient testing conditions were consistently kept at 21.3 ± 0.3 ºC and 43 ± 2% relative humidity.

Experimental protocol. During the experiment, participants were housed in a university residence in order to standardize resting times, nutrition, and training habits. One day before the first experimental trial, skinfold thickness was measured at four sites (biceps, triceps, subscapular and supra-iliac areas) on the right side of their body using a calibrated calliper (Holtain Ltd, Bryberian, Crymmych, Pembrokeshire). All anthropometric measurements were obtained by an ISAK certified anthropometrist (Durnin & Womersley, 1974; Marfell-Jones, Olds, Stewart, & Carter, 2006). The day before each trial, participants performed light and standardized training (<1 h in duration). In addition, 24 h before each trial participants kept a similar diet/fluid regime in order to avoid the effects of nutrition on the results of the investigation. Participants were also required to refrain from alcohol, caffeine and foods that contained *Citrus aurantium* (e.g., bitter orange, sweet orange and tangerine) for 24 h before each trial. Prior to the second trial, these standardizations were replicated.

On the day of each trial, participants arrived at the laboratory (08.00 am) in a fasted state (at least 8 hours after their last meal). Upon arrival, the assigned experimental capsule was provided in an unidentifiable bag and immediately ingested with water. Participants then dressed in a jersey, cycling shorts, cleated shoes, a heart rate belt (Wearlink, Polar, Finland) was attached to their chest, and then they rested in a supine position for 60 min. Resting heart rate and systolic and diastolic blood pressure (M6 Comfort, Omron, Japan; by triplicate) were measured during the last 5 minutes of the resting period. An average of three blood pressure measurements, performed with 1 min between repetitions, was used for analysis.

After the resting measurements, participants performed a standardized warm-up that included 10 min at 150 W on a cycle ergometer (SNT Medical, Cardgirus, Spain). They then completed a ramp exercise test on the cycle ergometer, which was comprised of 25 W increments every minute until volitional fatigue. The use of 1-min stages was selected to obtain maximal values during the test, especially regarding wattage and ventilatory variables. Expired gases were collected using a stationary breath-by-breath device (Metalyzer 3B, Cortex, Germany) during the test to calculate oxygen uptake (VO_2) and carbon dioxide production (VCO_2). A previous investigation using a bicycle ergometer ramp exercise test has measured an intraclass coefficient correlation of 0.969 and 0.964 for VO_2 and VCO_2, respectively (Meyer, Georg, Becker, & Kindermann, 2001). At each workload, gas exchange data was averaged every 15 s and the last 15 s of each stage was used as a representative value of the workload. At the end of the incremental exercise test, the workload imposed by the cycle ergometer was considered as the maximal wattage (Wmax) while VO_2max was defined as the as the highest VO_2 value obtained in any of the 1-min stages during the test (Edvardsen, Hem, & Anderssen, 2014). The exercise test was considered maximal and valid when the following end criteria were reached at the end of the test: VO_2 stabilized despite increases in ergometric power with a difference between the last two consecutive loads less than 0.15 L/min, the respiratory exchange ratio was higher than 1.10, and the participant's rating of perceived exertion (6-to-20 point Borg scale) was higher than 19 points while his heart rate was greater than 80% of the age-adjusted estimate of maximal heart rate (Edvardsen et al., 2014).

Certified calibration gases (16.0% O_2; 5.0% CO_2, Cortex, Germany) and a 3-L syringe were used to calibrate the gas analyser and the flowmeter before each trial. The same test with the identical workloads and times was used for the two experimental trials.

Participants chose a cadence between 70 and 90 rpm and it was recorded and replicated in both experimental trials.

Calculations. At the end of the test, Wmax values were comparable between *p*-synephrine and the placebo (458;441-475 vs 457;439-474 W, respectively; $P = 0.79$). For this reason, the absolute exercise intensity (in W) was normalized by using individuals' Wmax , following a previous investigation (Capostagno & Bosch, 2010). For this normalization, the wattage of each workload was divided by the individual Wmax, and the relative load (i.e., % of Wmax) was then allocated to the nearest load by using 5% intervals. Thus, the loads used in this investigation were: 28 ± 2; 34 ± 2; 40 ± 2; 45 ± 2; 51 ± 3; 57 ± 3; 62 ± 3; 67 ± 4; 74 ± 5; 79 ± 5; 85 ± 5; 91 ± 5; 95 ± 3; 97 ± 3; 100 ± 0% Wmax. We used Wmax as the normalizing variable instead of VO_2max because participants presented similar VO_2 values in the last 2-3 stages of the test due to their oxygen intake plateauing. Energy expenditure and substrate oxidation (fat and carbohydrate) rates were calculated using the non-protein respiratory quotient (Brouwer, 1957). Energy expenditure (kcal/min) during exercise was calculated as $(3.869 \times VO_2) + (1.195 \times VCO_2)$, where VO_2 and VCO_2 are in $L \cdot min^{-1}$. Fat oxidation rate (g/min) was calculated as $(1.67 \times VO_2) - (1.67 \times VCO_2)$ and carbohydrate oxidation rate (g/min) was calculated as $(4.55 \times VCO_2) - (3.21 \times VO_2)$. The maximal rate of fat oxidation (g/min) during exercise was individually calculated for each participant as the highest value of fat oxidation rate obtained during the ramp exercise intensity test. The exercise intensity at which maximal fat oxidation was achieved (Fatmax) was also obtained for each individual.

Statistical Analysis. Data was collected as previously indicated and the results of each test were blindly introduced into the statistical package SPSS v 20.0. The normality

of each quantitative variable was initially tested with the Shapiro-Wilk test. All the quantitative variables included in this investigation presented a normal distribution ($P > 0.05$) and parametric statistics were used to determine differences between *p*-synephrine and placebo interventions. Student's T tests for paired samples were used to compare heart rate, and systolic and diastolic blood pressures at rest. Student's T tests were also used to determine the effect of *p*-synephrine on the maximal fat oxidation rate and on Fatmax values. A two-way ANOVA (treatment × load) was used to compare energy expenditure, heart rate, and fat and carbohydrate oxidation rates obtained during the ramp test. After a significant F test (Geisser–Greenhouse correction for the assumption of sphericity), differences between means were identified using Tukey's post-hoc tests. The significance level was set at $P < 0.05$. All data are presented as means ± 95% of confidence intervals (CI).

RESULTS

Resting heart rate (mean; 95% of confidence intervals = 48; 44-52 vs 48; 43-53 beats/min, respectively; $P = 0.77$), systolic blood pressure (121; 116-126 vs 123; 119-127 mmHg; $P = 0.25$), and diastolic blood pressure (73; 69-77 vs 72; 70-74 mmHg; $P = 0.65$) were not different between experimental sessions.

During exercise, energy expenditure rate progressively increased with the exercise workload in both interventions (Figure 1; main effect for load F = 2,918, $P < 0.01$). However, the ingestion of *p*-synephrine did not modify the rate of energy expenditure at any workload (main effect of treatment F = 0.71, $P = 0.40$). During exercise, heart rate increased with the intensity (Figure 1; main effect for load F = 30.8, $P < 0.01$) but the

ingestion of *p*-synephrine did not produce any statistical difference from the placebo through the ramp test on heart rate (main effect for treatment F = 0.66, *P* = 0.43).

Figure 2 depicts the effects of *p*-synephrine on substrate oxidation during exercise. Overall, the relationship between fat oxidation rate and exercise intensity in both trials was explained by an inverted-U-shaped curve with peak values at moderate intensity exercise (main effect for load F = 121.9, *P* < 0.01). However, the pre-exercise ingestion of *p*-synephrine moved the fat oxidation rate-exercise intensity curve upwards (main effect for treatment F = 5.1, *P* = 0.04). In the pairwise comparisons between treatments, *p*-synephrine increased fat oxidation rate at 45 ± 2% Wmax (mean difference for *p*-synephrine-placebo pairwise comparisons; 95% of confidence intervals = 0.14; 0.11-0.17 g/min, *P* = 0.05), at 51 ± 3% Wmax (0.11; 0.08-0.15 g/min, *P* = 0.03), at 62 ± 3% Wmax (0.19; 0.14-0.24 g/min, *P* < 0.01), at 67 ± 4% Wmax (0.08; 0.05-0.11 g/min, *P* = 0.04), at 79 ± 5% Wmax (0.07; 0.04-0.10 g/min, *P* = 0.02) and at 85 ± 5% Wmax (0.11; 0.07-0.15 g/min, *P* = 0.03). The ingestion of *p*-synephrine did not modify the maximal rate of fat oxidation during the ramp test (0.91; 0.79-1.03 vs 1.01; 0.91-1.11 g/min, respectively, *P* = 0.06). Nevertheless, the intake of *p*-synephrine did not affect the exercise intensity at which the rate of maximal fat oxidation was obtained (i.e., Fatmax = 49; 47-51 vs 50; 48-53% Wmax, *P* = 0.52).

The rate of carbohydrate oxidation increased along the workload (Figure 2; main effect for load F = 265.0, *P* < 0.01). In addition, there was a main effect of *p*-synephrine on reducing carbohydrate oxidation (F = 4.8, *P* = 0.05). In the pairwise comparisons between treatments, *p*-synephrine reduced the rate of carbohydrate utilisation at 40 ± 2% Wmax (0.43; 0.23-0.63 g/min, *P* = 0.03), at 45 ± 2% Wmax (0.34; 0.19-0.49 g/min, *P* = 0.03) and at 62 ± 3% Wmax (0.45; 0.26-0.64 g/min, *P* = 0.05).

DISCUSSION

The objective of this research was to determine the substrate oxidation effects of *p*-synephrine during exercise in elite road cyclists. In this double-blind, placebo-controlled and well-standardized study, the main findings were: a) the ingestion of 3 mg of *p*-synephrine per kg of body mass did not produce any measurable effect on heart rate or blood pressure at rest; b) the ingestion of *p*-synephrine did not affect energy expenditure or heart rate during the ramp exercise test; c) *p*-synephrine moved upwards the relationship between fat oxidation rate and exercise intensity with statistical significant differences at loads between 45 and 85% of Wmax; d) *p*-synephrine did not modify the maximal fat oxidation rate obtained during exercise and it did not produce any measurable change in the exercise intensity that produced this event (i.e., Fatmax); e) *p*-synephrine also reduced the rate of carbohydrate oxidation at loads between 40 and 62% of Wmax. These results, when taken together, support the effectiveness of acute *p*-synephrine intake at increasing the rate of fat oxidation during exercise at low-to-moderate exercise intensities in well-trained athletes, as previously reported in healthy and active individuals (Gutierrez-Hellin & Del Coso, 2016, 2018a, 2018b). Interestingly, *p*-synephrine produced this effect on substrate oxidation without affecting Fatmax (Gutierrez-Hellin & Del Coso, 2016, 2018b) suggesting that the benefits of this phytochemical to enhance fat oxidation can be obtained during exercise training without modifying the exercise intensity.

Most of the studies that have investigated conditions to optimize fat oxidation during exercise have used exercise protocols of increasing intensity while measuring expired gases to calculate substrate oxidation by stoichiometric equations (Maunder et al., 2018). This test, first proposed by Achten et al. (2002) as the "Fatmax" test, defines the relationship

between exercise and fat oxidation rate across a range of exercise intensities. The Fatmax test allows the assessment of two main events: the maximal rate of fat oxidation rate and the intensity at which it occurs. Due to its increasing exercise intensity nature, the Fatmax test also allows for a clear identification of the efficacy of strategies used to increase fat oxidation in an ample range of exercise intensities. By using a modified version of this test, with 1-min stages, the current investigation suggests an effect of *p*-synephrine to increase whole-body fat utilisation at the expense of reduced carbohydrate oxidation (Figure 2). Although the effect of *p*-synephrine to shift the use of substrate during exercise was evident in elite cyclists, this alkaloid only produced statistically significant differences at moderate exercise intensity (45-to-85% of Wmax). Interestingly, the effect of *p*-synephrine on the maximal rate of fat oxidation during exercise of increasing intensity was not statistically significant from the placebo. Furthermore, the magnitude of this effect (e.g., +11.0 ± 1.9% respect to the placebo) was lower than the one found in healthy and active individuals (+38-57% respect to the placebo; (Gutierrez-Hellin & Del Coso, 2016, 2018a, 2018b)).

Randell et al. (Randell et al., 2017) measured maximal fat oxidation rates in an athletic population composed of 1,121 athletes from a variety of sports and competitive levels. They found that in men, maximal fat oxidation was ~0.60 g/min with a range between 0.17 and 1.27 g/min. They also found that the sport with the highest overall maximal rate of fat oxidation was rugby (i.e., ~0.72 g/min), likely due their high values of body mass and fat free mass. However, Randell et al. did not include athletes of endurance sports despite endurance training induces several adaptations linked to enhanced fat oxidation during exercise (Del Coso, Hamouti, Ortega, & Mora-Rodriguez, 2010). In the current investigation, carried out with elite cyclists, maximal fat oxidation was 0.91;0.79-1.03 g/min in the placebo situation, placing this sample of cyclists in the highest spectrum

of values found in athletes. In addition, the normalization of the peak of fat oxidation by fat free mass in kg further increases the differences between rugby (9.5 mg/kg fat free mass/min), found by Randell et al. (2017) and elite cyclist (13.7 mg/kg fat free mass/min) in the current investigation. To the authors' knowledge, the current investigation presents the highest values, as a group mean, for maximal fat oxidation rate during a ramp exercise test found in the literature and surpasses the normative values proposed for endurance trained males (Maunder et al., 2018). Of note, this value even increased with the ingestion of *p*-synephrine in this sample of elite cyclists.

p-Synephrine has been included in the Monitoring Program of the World Anti-Doping Agency (WADA) since 2005 due to is purported effect on increasing physical performance (World Anti-Doping Agency, 2018). It tracks the use of ergogenic substances that are not prohibited in- or out-of-competition but are still under anti-doping control until sufficient scientific or medical evidence indicates that they are safe/unsafe. However, the evidence to support *p*-synephrine's ergogenicity is scarce and contradictory. It has been found that acute *p*-synephrine intake might increase muscle endurance performance (Ratamess et al., 2015) and reduce perceived exertion during endurance exercise (Haller et al., 2008). Conversely, *p*-synephrine did not increase running velocity or jump performance in experienced sprinters during simulated competition (Gutierrez-Hellin et al., 2016). Although this was not the purpose of the current investigation, we indirectly assessed cyclists' physical performance because the end-point of the ramps tests was voluntary fatigue. The ingestion of *p*-synephrine did not modify the maximal wattage obtained in the test (458;441-475 vs 457;439-474 W) nor the VO_{2max} values (78.0 ± 3.53 vs 78.0 ± 2.79 mL/kg/min). Although these outcomes preclude the categorization of *p*-synephrine as an ergogenic aid, the higher fat oxidation rates at moderate exercise intensities -- at the

expense of lower carbohydrate use -- might help elite cyclists to spare muscle and liver glycogen in competition. The reasons for the reduction in carbohydrate oxidation in several exercise loads might be related to the downregulation of the enzyme pyruvate dehydrogenase kinase (Maldonado et al., 2018). However, the current data indicate that increase in fat oxidation rate was not always accompanied of a concomitant reduction in the rate of carbohydrate oxidation because a non-significant change in energy expenditure has been detected in several workloads. While muscle and liver glycogen sparing through carbohydrate feeding during exercise (Stellingwerff et al., 2007) or training with low carbohydrate availability (Hearris, Hammond, Fell, & Morton, 2018) has been found effective to increase cycling performance, the usefulness of the reduction on carbohydrate oxidation *p*-synephrine ingestion is still speculative and requires further investigation.

The safety of *p*-synephrine and bitter orange extracts has been frequently questioned, probably due to the structural similarities of this alkaloid with that of epinephrine and nor-epinephrine. Although some cardiovascular adverse effects have been reported when ingesting thermogenic supplements that contain *p*-synephrine or *Citrus aurantium* (Di Lorenzo et al., 2015), the adverse effects might be more related to other co-ingredients included in this type of supplements – such as caffeine – than to the isolated effects of *p*-synephrine (Vitalone et al., 2011). Recent reports have revealed that acute (Shara, Stohs, & Mukattash, 2016) and prolonged *p*-synephrine ingestion (Shara, Stohs, & Smadi, 2018) did not produce any cardiovascular side-effects. In addition, the prevalence of other side-effects such as headaches, abdominal discomfort and insomnia are not increased by *p*-synephrine (Gutierrez-Hellin et al., 2016). In the current investigation, *p*-synephrine did not modify blood pressure or heart rate at rest and it did not change heart rate at any workload during exercise. Increased fat oxidation without changes in heart rate have been found in previous

investigations with the same dose of *p*-synephrine (Gutierrez-Hellin & Del Coso, 2016, 2018b). This phenomena might be explained by the high binding of *p*-synephrine to β-3 adrenoreceptors and the of low binding of this alkaloid to β-1 and β-2 adrenoreceptors (Stohs & Preuss, 2012). This investigation adds information to claim the effect of *p*-synephrine to produce measurable effects on substrate oxidation during exercise without modifying cardiovascular response.

In the current investigation, we used synthetic *p*-synephrine, composed of a mixture of the enantiomeric compounds *l*- and *d*- (Mercader, Wanecq, Chen, & Carpéné, 2011). The *l*- form is found in bitter orange, while the *d*- form does not naturally occur in fruits (Pellati, Benvenuti, Melegari, & Firenzuoli, 2002; Pellati, Cannazza, & Benvenuti, 2010). It is well known that a mixture of these two enantiomers, as it happens with synthetic *p*-synephrine, might have less activity than the isolated intake *p*-synephrine obtained from bitter orange (Ma, Bavadekar, Schaneberg, Khan, & Feller, 2010). Thus, it is possible that the effect of *p*-synephrine on fat oxidation shown in this investigation might occur with lower doses if they are obtained directly from bitter orange (Sidney & Harry, 2012).

There are limitations associated with this study that must be considered in order to improve the results' applicability. First, we used a modified version of the traditional Fatmax test, with 1-min stages instead of the typical 3-min step. This modified version of the Fatmax test was performed to improve the identification of VO_{2max} and maximal wattage during the cycling protocol, especially in these highly trained individuals that completed 12-15 stages until fatigue. Future investigations should determine if *p*-synephrine exerts the same effect on substrate oxidation by using the traditional 3-min Fatmax protocol. In addition, the Fatmax test is a very useful tool to assess the maximal rate of fat oxidation and the exercise intensity at which it occurs in only one experimental

session, replacing the use of several experimental trials to assess these variables. However, the Fatmax test is not a typical training routine for cyclists since they usually undergo prolonged continuous or intermittent activities. The usefulness of *p*-synephrine to increase fat oxidation should be confirmed by using exercise routines more applicable to cycling such as prolonged and constant-intensity exercise, as recently investigated in healthy individuals (Gutiérrez-Hellín et al., 2019). A second limitation is that we utilized indirect calorimetry to measure whole body fat oxidation and without blood or tissue samples, we are unable to determine if *p*-synephrine has an effect on adipose tissue or intramuscular triacylglycerols. Finally, our investigation only measured *p*-synephrine's acute effect on fat oxidation while this effect has to be confirmed when *p*-synephrine is ingested chronically.

In summary, the acute ingestion of 3 mg/kg of *p*-synephrine increased fat oxidation rates in elite cyclists over a wider range of exercise intensities (from 45 to 85% Wmax). This shift in the substrate used for oxidation during exercise was presented without any cardiovascular modifications or energy expenditure changes that could negatively affect cycling performance. In addition, *p*-synephrine did not improve VO_{2max} nor maximal cycling power during the test but reduced the rate of carbohydrate oxidation at loads between 40 and 62% of Wmax. Thus, although *p*-synephrine cannot be considered as an ergogenic aid, the use of *p*-synephrine might offer some benefits for cyclists during exercise, such as body fat reduction, increased lipid utilization and glycogen sparing during competition. More translational research is necessary to practically apply this effect.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

All authors declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years; and no other relationships or activities that could appear to have influenced the submitted work.

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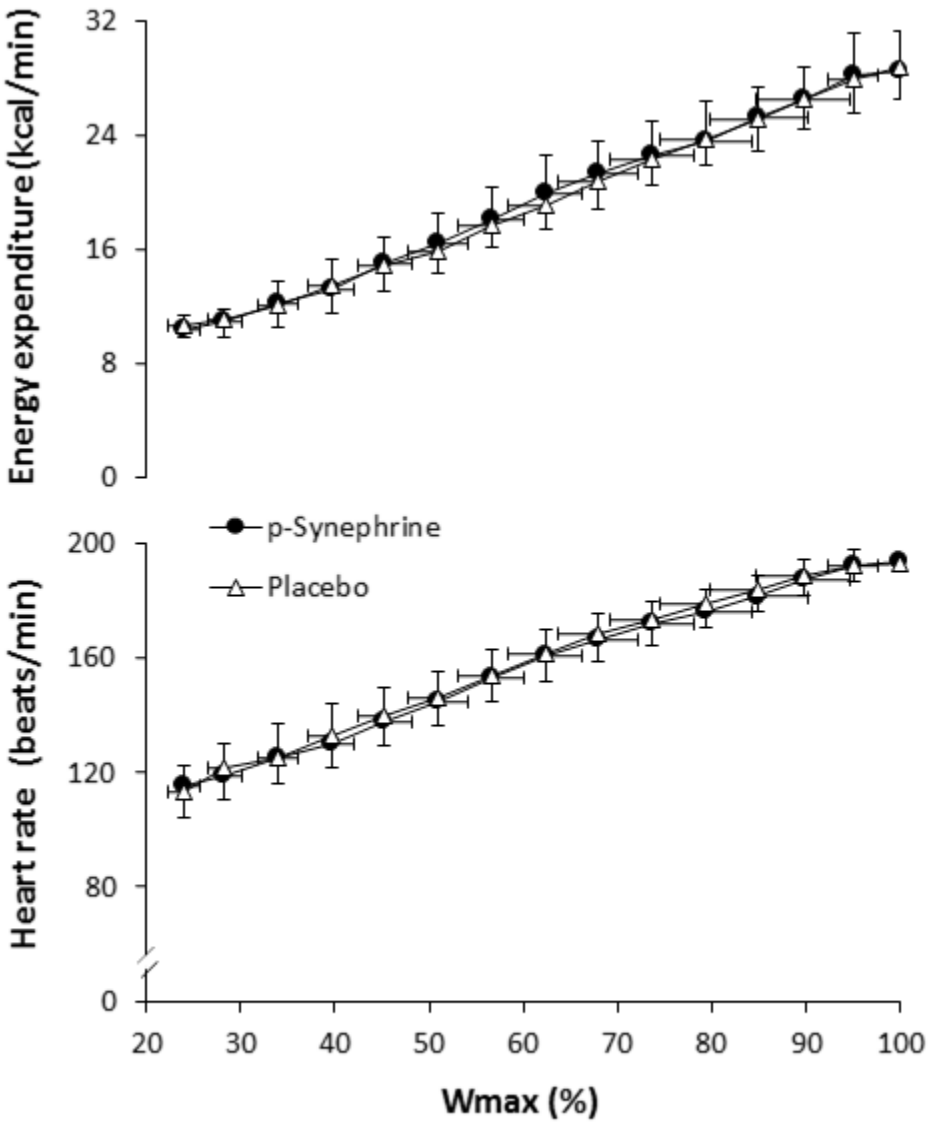
Figure 1. Energy expenditure (upper panel) and heart rate (lower panel) during exercise of increasing intensity 1 h after the ingestion of 3 $mg \cdot kg^{-1}$ of *p*-synephrine or a placebo. Data are mean ± 95 confidence intervals for 15 elite cyclists.

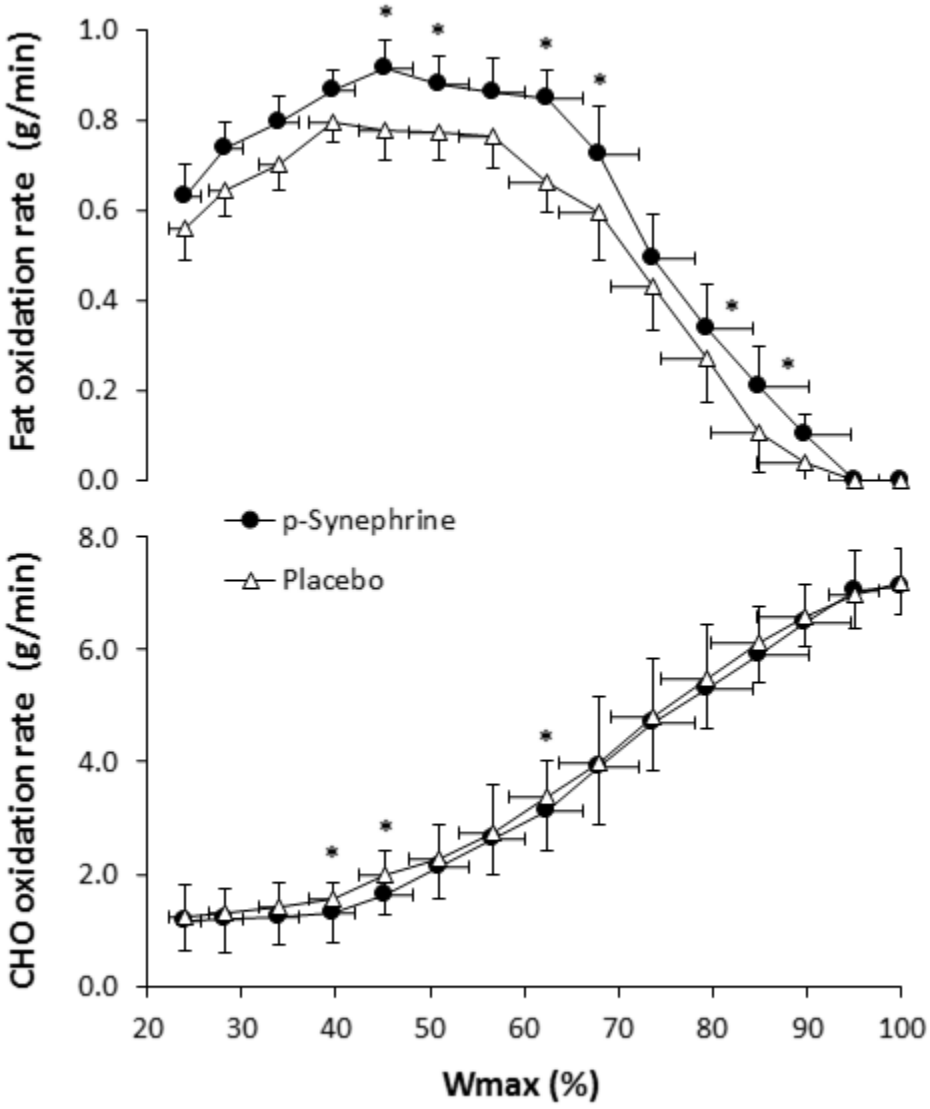
Figure 2. Fat (upper panel) and carbohydrate (CHO; lower panel) oxidation rates during exercise of increasing intensity one hour after the ingestion of 3 $mg \cdot kg^{-1}$ of *p*-synephrine or a placebo. Data are mean ± 95% confidence intervals for 15 elite cyclists.

(*) *p*-synephrine different from placebo at $P < 0.05$.

Table 1 Participants' age, morphological characteristics and maximal values at the end of a ramp test on a cycle ergometer. Data are presented as mean ± standard deviations (SD) with minimal and maximal values (range).

Variable (units)	**Mean ± SD**	**Range**
N	15	
Age (yr)	20.4 ± 1.1	19-22
Body mass (kg)	71.1 ± 6.6	58-79
Height (m)	1.78 ± 0.06	173-187
Fat mass (kg)	7.4 ± 1.9	4.1-10.4
Fat free mass (kg)	66.3 ± 5.3	53.7-70.5
VO_2max ($ml \cdot kg \cdot min^{-1}$)	77.8 ± 8.0	64.0-88.0
VO_2max ($l \cdot min^{-1}$)	5.5 ± 0.6	4.3-6.3
Maximal heart rate ($beat \cdot min^{-1}$)	195 ± 6	184-203
Maximal respiratory quotient	1.17 ± 0.09	1.12-1.34
Maximal Borg's scale rating (a.u.)	20 ± 0.2	19-20


Energy expenditure (kcal/min)
32
24
16
8
0
p-Synephrine
Placebo
Heart rate (beats/min)
200
160
120
80
0
20
30
40
50
60
70
80
90
100
Wmax (%)


Fat oxidation rate (g/min)
1.0
0.8
0.6
0.4
0.2
0.0
p-Synephrine
Placebo
CHO oxidation rate (g/min)
8.0
6.0
4.0
2.0
0.0
20
30
40
50
60
70
80
90
100
Wmax (%)