Acute *p*-synephrine ingestion increases whole-body fat oxidation during 1-h of cycling at Fatmax

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ABSTRACT

Purpose: *p*-Synephrine, the principal alkaloid of bitter orange (*Citrus aurantium*), is widely used in dietary supplements for weight loss due to its purported effect of increasing fat oxidation. However, there is a paucity of scientific information about its effectiveness in enhancing fat oxidation during exercise. The aim of this investigation was to determine the effect of an acute dose of *p*-synephrine on substrate oxidation during prolonged and constant intensity exercise. **Methods:** In a double-blind and randomized experiment, 14 healthy subjects performed two acute experimental trials after ingesting either *p*-synephrine (3 $mg \cdot kg^{-1}$) or a placebo (cellulose). Energy expenditure and fat oxidation rates were continuously measured by indirect calorimetry during 1 h of continuous cycling at Fatmax, the intensity that induces maximal fat oxidation rate. **Results:** In comparison to the placebo, energy expenditure during 1 h of cycling remained unchanged with *p*-synephrine (698±129 vs. 686±123 kcal, P=0.08). However, *p*-synephrine increased whole-body fat oxidation (33.6±10.4 vs. 37.3±9.8 g, P<0.01) while also reducing carbohydrate oxidation (99.5±30.4 vs. 85.0±28.4 g, P<0.01). However, the magnitude of the shift on substrate oxidation induced by *p*-synephrine was small. **Conclusion:** Acute ingestion of *p*-synephrine augments fat oxidation during prolonged and constant-intensity exercise.

Keywords: nutrition supplement; exercise; *citrus aurantium*; bitter orange; maximal fat oxidation.

INTRODUCTION

Several forms of exercise have been deemed as effective in inducing a permanent loss of body mass and a reduction in body adiposity [1]. However, exercise volume, frequency and intensity are key factors for the efficacy of exercise training in the prevention of weight gain, for weight loss, and for prevention of weight regain after weight loss [2]. While diet is a potent and operative strategy to reduce body mass and body fat, the addition of exercise to a diet increases the cardiovascular, metabolic, and body composition benefits of a weight loss program [3]. Dietary supplements are also used alone or in combination with exercise and diet to produce more effective body composition changes, but scientific literature that demonstrates the efficacy of most commercially-available dietary weight loss supplements is scarce. Overall, dietary supplements for weight management seek to increase energy expenditure and/or enhance fat oxidation at rest or during exercise [4]. While many botanical and other types of dietary supplements are sold under the premise of increasing energy expenditure or fat utilization, only caffeine has been systematically found as effective in increasing fat oxidation at rest [5] and during exercise [6].

p-synephrine, the most active substance in *citrus aurantium*, is a substance widely included in dietary supplements for weight loss [7]. It is often included in dietary supplements as a way to purportedly increase fat oxidation within the body. Its presence in the supplement market rose after the Food and Drug Administration (FDA) issued a final rule prohibiting the sale of dietary supplements containing ephedrine alkaloids in 2004. Concerns about the safety of *p*-synephrine were initially raised [8] but several most recent investigations have provided scientific evidence regarding the lack of acute and long-term side effects derived from *p*-synephrine intake [9]. Nevertheless, the effectiveness of *p*-synephrine at enhancing fat oxidation has only been found in a few

investigations [6, 10, 11]. In these investigations, *p*-synephrine (at a dose of 2-3 $mg \cdot kg^{-1}$) enhanced whole-body fat oxidation at low-to-moderate exercise intensity, while this change in substrate oxidation was identified by using a short-time exercise protocol of increasing intensity that allows the assessment of fat oxidation rates at several exercise intensities in only one experimental session. However, this ramp exercise test used in previous research has little applicability to exercise training for weight loss because the duration (12-21 min) impedes high values of energy expenditure or fat oxidized. To the date, there is no investigation that have studied the effect of *p*-synephrine during prolonged continuous exercise, despite this training routine is typically used in weight loss programs [1].

Due to the current body of scientific evidence, it is difficult to ascertain whether *p*-synephrine is an effective substance to increase the amount of fat utilized during an exercise training session. For this reason, the aim of this research was to determine the effect of an acute ingestion of *p*-synephrine on substrate oxidation during 1 h of steady-state cycling at the intensity of maximal fat oxidation.

METHODS

Subjects. Fourteen young and healthy participants volunteered to participate in this investigation (age= 31±6.9 years, body mass= 71.0±5.8 kg, height= 1.76±0.04 m, body mass index= 22.7±1.8 $kg \cdot m^2$, maximal oxygen uptake [VO_{2max}] = 56.7±10.6 $mL \cdot kg^{-1} \cdot min^{-1}$). There were two women in the sample that performed all the experimental trials in their luteal phase. One week before the study's onset, participants were fully informed of the experimental standards and risks associated with the research. Participants signed an informed written consent form to participate in the investigation. The study was

89 approved by the Camilo José Cela University Research Ethics Committee, in accordance
90 with the Declaration of Helsinki.

91 **Experimental design.** A double-blind, placebo-controlled, and randomized
92 experimental design was used in this investigation. Each participant took part in 2
93 experimental trials separated by 3 days to allow for complete recovery and substances
94 elimination. Participants either ingested 3 mg of *p*-synephrine per kg of body mass (99%
95 purity; Synephrine HCL, Nutrition Power, Spain) that was contained in an opaque capsule
96 or an identical capsule filled with a placebo (100% cellulose, Guinama, Spain). The
97 capsules were ingested with 150 mL of tap water 60 min before the onset of the
98 experimental trials. An alphanumeric code was assigned to each trial by a person
99 independent of the study in order to blind the participants and investigators to the tested
100 substances. Environmental temperature and humidity (mean ± standard deviation was
101 21.2±0.4 ºC for air temperature and 43±9% for relative humidity) were recorded using a
102 digital temperature and humidity monitor (OH1001, OH Haus, Spain).

103 **Pre-experimental procedure.** One week before the first experimental trial,
104 participants underwent standardized warm-up that included 10 min at 50 W on a cycle
105 ergometer (SNT Medical, Cardgirus, Spain). They then completed a ramp exercise test
106 on the cycle ergometer, which was comprised of 25 W increments every 3 minutes until
107 volitional fatigue. During the test, participants chose a cadence between 70 and 90 rpm
108 and the test was finished when participants were unable to maintain a cadence > 50 rpm.
109 During the incremental exercise test, oxygen uptake (VO_2) and carbon dioxide production
110 (VCO_2) were measured through indirect calorimetry. Participants were continuously
111 measured by a breath-by-breath analyzer (Metalyzer 3B, Cortex, Germany) to calculate
112 fat oxidation and carbohydrate oxidation rates at each stage. The exercise intensity in
113 which the maximal rate of fat oxidation was achieved (Fatmax; mean ± standard deviation

was 147 ± 39 W) was registered and used for the subsequent experimental trials. The ramp exercise test was considered maximal and valid when the end criteria for VO_{2max} were reached at the end of the test: VO_2 stabilization despite increases in ergometric power, the respiratory exchange ratio was higher than 1.10, the participant's rating of perceived exertion -- measured with the 6-to-20-point Borg scale-- was higher than 19 points while participants' heart rate was superior to 80% of the age-adjusted estimate of maximal heart rate [12]. On the subsequent day, a familiarization protocol as described below was performed on all individuals.

Experimental procedures. Twenty-four hours before each experimental trial, participants refrained from strenuous exercise and adopted a similar diet and fluid intake regimen. Subjects were also required to refrain from consuming alcohol, caffeine, and foods that contained *citrus aurantium* (e.g., bitter orange, sweet orange and tangerine) for 24 h before each trial. To standardize these routines, subjects were requested to complete a 24-h dietary record on the day before the first trial and to follow the same dietary pattern before the second visit. On the day of the experimental trials, participants arrived at the laboratory (09.00 am) in a fasted state (at least 8 hours after their last meal). Upon arrival, the capsule with the assigned experimental treatment was provided in an unidentifiable bag and then later ingested by the participant. Then, participants rested supine for 60 min to allow for substance absorption. Resting heart rate and systolic and diastolic blood pressure (M6 Comfort, Omron, Japan; by triplicate) were measured during the last 5 min of the resting period. An average of three blood pressure measurements was used for analysis.

After the resting measurements, participants performed a standardized warm-up that included 10 min on the same cycle ergometer used for the ramp test. During the warm-up, exercise intensity was progressively increased until reaching individual Fatmax as

measured in the pre-experimental trial (equivalent to 57.1±6.4% of VO_{2max}). At the end of the warm-up, participants completed 1 h of constant exercise at Fatmax. During the whole trial, heart rate (Wearlink, Polar, Finland) and gas exchange data were obtained – as previously described for the incremental exercise test – and averaged every 5 minutes in order to achieve a representative value. In the first experimental trial, pedaling cadence was freely chosen between 70 and 90 rpm, but it was recorded at 5 min intervals and replicated in the second trial. An average for the rate of substrate oxidation was calculated in these 5-min periods and the amount of fat and carbohydrate oxidized were also calculated for the whole trial. The same procedures were used for the two experimental trials.

Statistical Analysis. The results of each test were blindly introduced into the statistical package SPSS v 20.0 and analyzed afterwards. 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 and parametric statistics were used to determine differences among trials. A one-way analysis of variance (ANOVA) was used to compare heart rate and blood pressure at rest. A two-way ANOVA (treatment × time) was used to compare the variables obtained during exercise. After a significant F test (Geisser–Greenhouse correction for the assumption of sphericity), differences between means were identified using Tukey's post- hoc tests. Only the main effects for substance are presented for clarity. Paired t-tests were used to compare total energy expenditure and total fat and carbohydrate oxidation in the *p*-synephrine and placebo trials. The significance level was set at $P < 0.05$. The data are presented as mean±standard deviation. The effect size (±90% confidence intervals (CI)) was calculated in all pairwise comparisons.

RESULTS

In comparison to the placebo, the ingestion of *p*-synephrine did not modify resting heart rate nor systolic, diastolic, and mean arterial blood pressure (Table 1). During exercise, there was no main effect of *p*-synephrine on heart rate ($P = 0.13$) and exercise heart rate was unaffected by the ingestion of *p*-synephrine at any time point. Similarly, there was no main effect of *p*-synephrine on the rate of energy expenditure ($P = 0.11$) and the rate of energy expenditure was unaffected by the ingestion of *p*-synephrine at any time point during 1-h exercise trial. The total energy expended during the trial was very comparable between substances (Table 1).

There was a main effect of *p*-synephrine on fat oxidation rate ($P < 0.01$) while the pairwise comparison indicated that fat oxidation was higher with *p*-synephrine than with placebo from min-25 to min-60 of the 1-h cycling trial (all $P < 0.05$, likely-most likely, Figure 1). As a result, total fat oxidation was higher with *p*-synephrine than with placebo (Table 1). There was a main effect of *p*-synephrine on carbohydrate oxidation rate ($P < 0.01$) and the pairwise comparison indicated that *p*-synephrine reduced carbohydrate utilization at min-5, min-20, and from min-30 to the end of the 1-h cycling trial (all $P < 0.05$, likely-most likely, Figure 1). As a result, total carbohydrate oxidation was reduced in comparison the placebo (Table 1). Figure 2 depicts the individual responses to *p*-synephrine ingestion on substrate oxidation during exercise. Out of the 14 participants, 11 (79%) presented a lower amount of carbohydrate oxidized with *p*-synephrine while 12 participants (86%) increased the amount of fat oxidized during the 1-h cycling trial with *p*-synephrine.

DISCUSSION

This investigation is novel because it is the first experiment that assesses the effect of acute *p*-synephrine intake on fat oxidation during a protocol of continuous exercise

that simulates an exercise session. This investigation is also valuable because this substance is commonly included in dietary supplements to reduce body fat and body mass without a proper scientific background to support its effectiveness [7]. The main results of this investigation suggest that 3 $mg \cdot kg^{-1}$ of *p*-synephrine might increase the amount of fat oxidized by 11.1±10.5% in 1 h of exercise at Fatmax, without affecting total energy expenditure or exercise heart rate. *p*-Synephrine was also accompanied by a reduction of carbohydrate utilization by 13.5±13.3%. Taken together, acute *p*-synephrine intake might be effective at producing a moderate shift towards enhanced utilization of fat during continuous steady-state exercise.

In animal models, it has been found that *p*-synephrine can bind to β-3 adrenergic receptors, resulting in enhanced lipid metabolism [7, 13]. Although the evidence is still scarce, a few investigations in humans have found that *p*-synephrine can potentially induce changes in substrate oxidation at rest and during exercise [6, 10, 11, 14]. To this regard, it has been suggested that activation of β-3 adrenoreceptors by *p*-synephrine might be responsible for the enhanced fat oxidation found in humans [15] although this hypothesis still requires confirmation. Due to the structural similarities of *p*-synephrine to that of epinephrine and nor-epinephrine, concerns have been raised regarding this substance's safety [8]. However, the binding of *p*-synephrine to α-, β-1 and β-2 adrenergic receptors is low, which explains its non-effect in causing cardiovascular effects -- even after 15 days of continuous ingestion [13]. This selective affinity of *p*-synephrine to adrenergic receptors means that this substance has a comparable effect to caffeine in increasing fat oxidation during exercise, but without the caffeine-induced effects on blood pressure [6]. These evidences for efficacy and safety of *p*-synephrine support the use of this substance for weight management although additional human studies are required to determine long-term safety and efficacy.

Although the acute pre-exercise intake of *p*-synephrine significantly increased the amount of oxidized fat during 1 h of cycling, its effect was small. In absolute terms, *p*-synephrine augmented the utilization of fat by 3.7±3.3 $g \cdot h^{-1}$ -- equivalent to 0.06 $g \cdot min^{-1}$ for the duration of the trial. The increase in fat oxidation induced by *p*-synephrine in the current investigation was slightly inferior to the ones found during exercise of increasing intensity (from 0.11 to 0.20 $g \cdot min^{-1}$) with the same dose [10, 11]. The difference among investigations might be related to the different of tests used (constant cycling at Fatmax vs increasing exercise intensity cycling test). In any case, this and previous investigations suggest the usefulness of *p*-synephrine in increasing the usage of fat during aerobic exercise. Interestingly, in all these investigations, the effect of *p*-synephrine on substrate oxidation was never accompanied by any change on energy expenditure. This also suggests that this substance cannot be considered as thermogenic.

In summary, pre-exercise ingestion of 3 mg of *p*-synephrine per kg of body mass was effective at producing a shift on substrate oxidation during 1 h of continuous cycling at Fatmax. With the ingestion of this protoalkaloid, the utilization of fat was enhanced at the expense of carbohydrate oxidation. This investigation is a step forward at confirming the efficacy of *p*-synephrine in modulating substrate utilization during exercise -- although this effect is of a small magnitude. Nevertheless, further experiments are needed to ascertain tolerance to this substance and its real contribution at enhancing reductions in body fat during weight loss programs. Lastly, the outcomes found in this investigation are not transferable to bitter orange or *citrus aurantium* dietary supplements because these natural compounds contain more substances than *p*-synephrine and the concentrations of this alkaloid are typically lower than the one used in the current investigation.

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CONFLICT OF INTEREST

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

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This investigation did not receive any funding.

AUTHOR CONTRIBUTIONS

Jorge Gutiérrez-Hellín[1,2,3,4,5], Carlos Ruiz-Moreno[1,2,3,6] and Juan Del Coso[1,2,3,4,6].

1. Formulated the research question
2. Designed the study
3. Conducted it
4. Analyzed the data
5. Wrote the article
6. Revised the article

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TABLES

Table 1. Metabolic and cardiovascular responses at rest and during 1 h of cycling at Fatmax after the ingestion of *p*-synephrine or a placebo.

Data is shown as mean±SD for 13 healthy participants.

Variables (Units)	Placebo	*p*-Synephrine	Effect size (±90% CI)	Qualitative inference	*P* value
Resting heart rate (beats/min)	50±8	50±8	0.0 (-0.3 / 0.3)	Unclear	0.84
Resting systolic blood pressure (mmHg)	118±8	118±7	0.0 (-0.3 / 0.2)	Unclear	0.80
Resting Diastolic blood pressure (mmHg)	70±8	68±8	-0.2 (-0.5 / 0.1)	Possible	0.19
Mean arterial blood pressure (mmHg)	86±7	85±6	-0.2 (-0.4 / 0.1)	Possible	0.29
Total fat oxidation (g)	33.6±10.4	37.3±9.8	0.3 (0.2 / 0.5)	Likely	<0.01
Total carbohydrate oxidation (g)	99.5±30.4	85.0±28.4	-0.5 (-0.7 / -0.2)	Likely	<0.01
Total energy expenditure (kcal)	698±129	686±123	-0.2 (-0.3 / 0.1)	Most unlikely	0.08
Average heart rate (beats/min)	127±12	126±12	0.1 (-0.2 / 0.4)	Unclear	0.63
Average respiratory exchange ratio	0.86±0.04	0.84±0.04	-0.6 (-0.8 /-0.3)	Very likely	<0.01

FIGURES

Figure 1. Carbohydrate (upper panel) and fat (lower panel) oxidation rates during 1 h of cycling at Fatmax after the ingestion of *p*-synephrine or a placebo.

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

Figure 2. Individual responses for carbohydrate (upper panel) and fat (lower panel) oxidation rates during 1 h of cycling at Fatmax after the ingestion of *p*-synephrine or a placebo.

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