Caffeine increases exercise intensity and energy expenditure but does not modify substrate oxidation during 1 h of self-paced cycling

Running head: **Caffeine and self-paced cycling**

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ABSTRACT

Aim: Oral caffeine intake has been deemed as an effective supplementation strategy to enhance fat oxidation during aerobic exercise with a steady-state intensity. However, in real exercise scenarios, individuals habitually train with autoregulation of exercise intensity. This study aimed to analyze the effect of oral caffeine intake during self-paced cycling on autoregulated exercise intensity and substrate oxidation. **Methods:** Fifteen young and healthy participants (11 men and 4 women) participated in a double-blind, randomized, cross-over investigation. Each participant took part in two experimental days consisting of pedaling for 1 h with a self-selected wattage. Participants were told that they had to exercise at a moderate intensity to maximize fat oxidation. On one occasion participants ingested 3 mg/kg of caffeine and on the other occasion ingested a placebo. Energy expenditure, fat oxidation rate, and carbohydrate oxidation rate were continuously measured during exercise by indirect calorimetry. **Results:** In comparison to the placebo, caffeine intake increased the self-selected wattage (on average, 105±44 vs 117±45 W, respectively, $P < 0.001$) which represented a higher total work during the cycling session (377±157 vs 422±160 kJ, $P < 0.001$). Caffeine increased total energy expenditure (543±161 vs 587±155 kcal, $P = 0.042$) but it did not affect total fat oxidation (24.7±12.2 vs 22.9±11.5 g, $P = 0.509$) or total carbohydrate oxidation (87.4±22.4 vs 97.8±32.3 g, $P = 0.101$). **Conclusion:** Acute caffeine ingestion before an exercise session with an individual's freedom to regulate intensity induces a higher self-selected exercise intensity and total work. The selection of a higher exercise intensity augments total energy expenditure but eliminates the effect of caffeine on substrate oxidation during exercise.

Keywords. Aerobic exercise, endurance exercise, dietary supplement, fat loss, weight loss.

INTRODUCTION

Beyond the well-supported effect of oral caffeine intake to enhance sports performance [1–3], this substance can exert other potential benefits during prolonged aerobic exercise of low-to-moderate intensity such as increased fat utilization [4]. The influence of caffeine to increase fat utilization during exercise may be an attractive effect for those individuals enrolled in exercise programs seeking body weight reduction and fat mass loss. The higher fat oxidation rate during exercise found after caffeine intake [4–7] may aid to produce a faster reduction of fat mass, as caffeine induces a higher amount of fat oxidized per exercise session [5]. However, this substance should be ingested chronically before the exercise sessions of a weight loss program to produce measurable changes in body composition as caffeine increases fat utilization in the range of 0.08 to 0.14 g/min -equivalent to 4.8-8.4 g of "extra" fat utilized per exercise session of 60 min of duration- [4–7].

The evidence supporting the effect of acute caffeine intake to enhance fat oxidation during prolonged exercise goes back to the '70s [8–10] but, still today, there are doubts about caffeine's efficacy to modify substrate use during exercise and how to use caffeine supplementation to obtain such benefit. These doubts arise because the effect of caffeine on fat oxidation during exercise has been found in some investigations [4, 5, 8–10] but not in others [11, 12]. A potential reason for the lack of agreement among investigations is the characteristics of the exercise protocols used to assess the effect of caffeine on fat oxidation. Exercise intensity is the main factor affecting fat oxidation rate during aerobic exercise [13] and caffeine can increase both, exercise intensity through a reduction of perceived fatigue and pain [14] and fat oxidation rate [5]. In investigations that used steady-state exercise intensity protocols, caffeine was habitually effective to enhance fat oxidation. On the other hand, the investigations that

employed exercise protocols with free-chosen exercise intensity the effect of caffeine on substrate oxidation was not evident.

In this context, the effect of acute caffeine intake (from 2 to 7 mg/kg of body mass) to enhance fat oxidation during aerobic exercise has been recently confirmed with a meta-analytic approach when limiting the analysis to studies that employed an exercise protocol with steady-state intensity [6]. However, in real exercise scenarios, individuals habitually train with autoregulation of exercise intensity. Hence, the effect of caffeine to increase exercise intensity during self-paced exercise (*i.e.,* ergogenic effect) may mitigate or even eliminate the effect of this substance on fat utilization. To date, the effect of oral caffeine intake during exercise with an individual's freedom to regulate intensity on substrate oxidation has not been investigated, at least in a context unliked to exercise performance. This study aimed to analyze the effect of oral caffeine intake during 1 h of self-paced cycling of moderate intensity on wattage, energy expenditure and substrate oxidation rates. We hypothesized that participants would cycle at a higher wattage during exercise in the caffeine trial which would offset the potential benefits of caffeine on increasing total fat oxidation.

METHODS

Participants: Fifteen young and healthy participants (11 men and 4 women) volunteered to participate in the study (age = 29±6 years, body mass = 72.2±9.4 kg, height= 1.76±0.09 m, peak oxygen uptake [VO_{2peak}] = 49.3±10.4 mL/kg/min). Women performed all experimental trials during the luteal phase of their menstrual to standardize data collection. An *a priori* sample size calculation indicated that at least 7 participants were required to obtain statistically significant differences between caffeine and placebo on total fat oxidation during 1 h of cycling. The required sample size was

calculated to obtain an effect size of 0.68 Cohen's *d* units based on a previous study that reported an increase of such magnitude in total fat oxidation with 3 mg/kg of caffeine [15]. The sample size was calculated using the G*Power software (v.3.1.9.7, Germany) with a statistical power of 0.80 and a two-tailed α level of 0.05, one group of participants and for ANOVA of repeated measures, within factors. The inclusion criteria to include participants in the study were: (a) regularly performing aerobic exercise training 1 hour per day, at least 4 days per week (b) age between 18 and 40 years (c) low caffeine intake as defined by Filip [16] (d) in women, regular duration of their menstrual cycle for the previous 4 months (My calendar®, Simpleinnovation, United States). Exclusion criteria were: (a) smoking status (b) using medications or any type of dietary supplementation in the month before testing (c) using oral contraceptives (d) allergy to caffeine (f) any cardiopulmonary or musculoskeletal disease. Before enrolment in the study, all potential participants were fully informed of the experimental procedures and potential risks associated to the experiment. Once inclusion/exclusion criteria were applied, all participants of the study provided oral and written informed consent. The study was approved by the Camilo José Cela University Research Ethics Committee and was conducted under the latest version of the Declaration of Helsinki.

Experimental Design: A double-blind, placebo-controlled, randomized, experimental design was used in this investigation. Each participant performed two identical experimental trials with at least 3 days between trials. In each experimental trial participants ingested either (a) 3 mg of caffeine per kg body mass (Bulk Powders, United Kingdom) or (b) 3 mg/kg body mass of an inert placebo substance (cellulose, Guinama, Spain). The substances were ingested in an opaque capsule with 150 mL of water. After ingestion, participants rested for 60 min and then completed 1 h of cycling with autoregulation of exercise intensity. Participants were informed that they had to exercise at moderate intensity to maximize the amount of fat oxidized during the

exercise session. Participants had been previously lectured about the association between exercise intensity, maximal fat oxidation (MFO) to decontextualize the effect of caffeine on exercise performance. Trials in which the respiratory exchange ratio surpassed 1.0 were removed from the analysis. To ensure the double-blind nature of the experiment, an alphanumeric code was designated to each trial by an independent researcher and experimenter and participants were not informed about the substance under investigation in each trial. Additionally, the experimenter and participants did not have access to workload and substrate oxidation data during the trials to avoid the influence of this information in the autoregulation of exercise intensity. All trials were carried out in an exercise physiology laboratory with controlled ambient temperature (20.3 ± 0.4º C) and relative humidity (31 ± 11%).

Pre-experimental trials: Participants performed two pre-experimental trials: in the first pre-experimental day, carried out one week before the onset of the experiment, participants performed an incremental test to measure VO_{2max}, MFO and Fatmax. The test was performed on a cycle ergometer (SNT medical, Cardgirus, Spain) and the initial workload was set to 75W for men and to 50W for women. After 10-min of warm-up with this workload, exercise intensity was increased by 25W for men and by 15W for women every three minutes until their respiratory exchange ratio reached 1.0. After this, the workload was increased by 25/15W for men/women each minute of exercise until exhaustion. Participants were instructed to maintain a cadence of 70-90 rpm throughout the test. During the test, oxygen consumption and carbon dioxide production were monitored breath-by-breath with a gas analyzer (Metalizer 3B, Cortex, Germany). Fat and carbohydrate oxidation rates were measured and calculated at each stage using the stoichiometric equations [17, 18]. The VO_{2max} test was considered valid when it met standard end criteria [19]. On this day, participants were lectured about the concepts of MFO, Fatmax and the effect of caffeine on these variables. The second pre-

experimental day was performed the following day and consisted of a familiarization trial replicating the procedures to be performed in the subsequent experimental trials, as explained below. In this second pre-experimental testing, participants fixed the saddle and handlebar position of the cycle ergometer and wore the same clothing that would be used in the trials. Participants were told that they had to pedal at moderate intensity to maximize fat and avoid pedaling as fast as they could. On this day, they could visualize workload and they knew the wattage that corresponded to their individual Fatmax.

Experimental trials: On the day of the experimental trials, participants arrived at the laboratory at (9.00 AM) in a fasting state (at least 8 h since their last meal). They were then given an opaque capsule containing caffeine or placebo and the experimenter verified that the participant ingested the capsule. After ingestion, participants lay supine for 60 minutes on a stretcher to allow substance absorption. Then, resting blood pressure (M6 comfort, Omron, Japan; by triplicate) and resting heart rate (Wearlink + V800, Polar, Finland; for 5 min) were measured. After the resting measurements, participants were weighed without any clothing (± 50 g, Radwag, Poland) and then rode the cycle ergometer to complete a 10-min warm-up of a light intensity (~50 W). After the warm-up, the cycle ergometer was set to produce a workload equivalent to their Fatmax, measured in the pre-experimental trial (105 ± 44 W). Participants cycled for 5 min at Fatmax and then participants completed the 1-h cycling session with the possibility of self-selecting the workload at 5-min intervals (the cycle ergometer was set to produce changes of ± 5 W in the resistance applied to pedaling with a button placed in the handlebar). Participants were able to change exercise intensity at 5-min intervals because, in the last minute of each 5-min interval, VO_2 and VCO_2 were measured with the same analyzer used for VO_{2max} testing and heart rate was recorded. Additionally, during this last minute of each 5-min interval, participants were encouraged to maintain a stable position on the cyclergometer and to maintain a stable cadence. This protocol

was set to produce representative values of VO_2, VCO_2 and heart rate for each 5-min period. During the test, participants only had information about the time left to complete the 1 h of cycling while data on wattage, heart rate and gas exchange data were hidden from the view of the participant and the experimenter. Rates of energy expenditure and substrate oxidation were calculated from VO_2 and VCO_2 using the non-protein respiratory quotient [17, 18]. VO_2 and VCO_2 data from the entire test (i.e., all breaths included in the 1-h cycling test) were used for the calculation of the total amount of energy expended and the total amounts of fat and carbohydrate oxidized. At the end of each 5-min interval, the rating of self-perceived fatigue was obtained with the 6-to-20-point Borg scale [20]. Once participants finished the exercise, nude body mass was measured again to calculate sweat rate, as the change in body mass was divided by exercise time. Then, participants continued with their daily activities but were encouraged to avoid any dietary source of caffeine and alcohol. Twenty-four hours after the end of the exercise, participants answered a questionnaire on side effects typically associated with caffeine intake. The questionnaire included a 1-10 point scale for each item and it has been previously used in athletes to assess the magnitude of the side effect associated with caffeine supplementation [21].

Standardizations: All participants included in this study were informed of various obligations they had to fulfill the day before the experimental trials: (a) avoid any type of food containing caffeine (*i.e.*, coffee, tea, chocolate, energy drinks, yerba mate) (b) avoid consumption of alcoholic beverages (c) maintain an 8-hour sleep pattern (d) avoid strenuous exercise (e) replicate meals and dinners (f) maintain euhydration. For this, standard fluid and diet guidelines [22, 23] were given to assure carbohydrate bioavailability and euhydration in all experimental trials. All dietary and exercise patterns were recorded in a diary the first experimental trial for replication on the second day of the experimental trial. Euhydration was measured at the participant's

arrival at the laboratory by collecting a urine sample which was used to assure that urine specific gravity, measured by refractometry (MASTER-SUR/N, Atago, Japan), was < 1.020 [24].

Statistical analysis: The study data were blindly introduced into the statistical package SPSS (SPSS, v. 22.0, IBM SPSS Statistics, IBM Corporation) and subsequently analyzed. The Shapiro-Wilk test was used to confirm the normality of the quantitative variables and, consequently, parametric statistics tests were used to determine differences among trials. A two-way analysis of variance (ANOVA) (substance × time; 2×12) was performed to analyze the effects of caffeine on all the variables under investigation. Sphericity assumption was checked with Mauchly´s test. In the case of a main effect of the substance, time or interaction between these two factors, pairwise comparisons between caffeine and placebo trials were performed at each 5-min interval and differences were identified with LSD post-hoc tests. Paired t-tests were used to detect differences in the caffeine-placebo comparison for resting heart rate, resting blood pressure variables, total energy expenditure, total fat and carbohydrate oxidation, and ratings of side effects post-exercise. In all statistical tests, a significance level of $P < 0.050$ was set to consider the difference as significant. The data are presented as mean ± standard deviation.

RESULTS

In comparison to the placebo, the ingestion of caffeine increased systolic blood pressure, diastolic blood pressure and mean arterial pressure at rest (Table 1; $P < 0.050$), while it did not modify resting heart rate ($P = 0.172$).

During exercise, there was a main effect of substance on exercise workload ($F_{1,11} = 7.95$; $P = 0.014$) with no effect of time ($F_{1,11} = 0.61$; $P = 0.767$) or substance × time interaction ($F_{1,11} = 0.80$; $P = 0.656$) in this variable. The post hoc analysis

229 revealed that participants selected a higher workload at all time points with caffeine in
230 comparison to the placebo (Figure 1a; $P < 0.050$). As a result, the total work performed
231 during the exercise session was higher with caffeine than with placebo (Table 1; $P <$
232 0.001). There was a main effect of substance on energy expenditure rate ($F_{1,11} = 4.99$; P
233 = 0.042) with no effect of time ($F_{1,11} = 3.80$; $P = 0.103$) or substance × time interaction
234 ($F_{1,11} = 1.48$; $P = 0.375$). The post hoc analysis revealed that participants had a higher
235 energy expenditure rate at 25, 30, 35, 55 and 60 min of exercise with caffeine in
236 comparison to the placebo (Figure 1b; $P < 0.050$). As a result, total energy expenditure
237 for 1 h of self-paced cycling was higher with caffeine than with the placebo (Table 1; P
238 = 0.042).

239 There was no main effect of substance on fat oxidation rate ($F_{1,11} = 0.81$; $P =$
240 0.382) but it was a main effect of time ($F_{1,11} = 23.20$; $P = 0.004$). The interaction
241 between these two variables did not reach statistical significance for fat oxidation rate
242 ($F_{1,11} = 0.31$; $P = 0.942$). The post hoc analysis revealed no caffeine-placebo
243 differences in fat oxidation rate at any time point (Figure 1c) and total fat oxidation
244 during the exercise session was similar with both treatments (Table 1; $P = 0.509$). The
245 was no main effect of substance ($F_{1,11} = 2.94$; $P = 0.107$), time ($F_{1,11} = 3.01$; $P = 0.114$)
246 nor interaction ($F_{1,11} = 2.14$; $P = 0.207$) on carbohydrate oxidation rate (Figure 1d). The
247 total amount of carbohydrate oxidized during exercise was similar with caffeine and
248 with placebo (Table 1; $P = 0.101$).

249 There was no main effect of substance on heart rate ($F_{1,11} = 1.17$; $P = 0.303$) and rating
250 of perceived exertion ($F_{1,11} = 0.90$; $P = 0.768$) while the main effect of time and
251 substance × time interaction on these variables was not present (Figure 1e and f). The
252 differences in sweat rate between caffeine and placebo trials did not reach statistical
253 significance (Table 1; $P = 0.066$).

For the twenty-four hours after exercise, participants reported higher ratings of nervousness (Table 2; $P = 0.033$), vigor ($P = 0.055$), diuresis ($P = 0.009$) and insomnia ($P = 0.035$) with caffeine than with the placebo.

DISCUSSION

This research is the first to analyze the effects of a moderate dose of caffeine (*i.e.*, 3 mg/kg of body mass) on energy expenditure and substrate oxidation during self-paced exercise in a context unrelated to exercise performance. The main findings of this research indicate that, in comparison to the placebo trial, oral ingestion of caffeine before exercise (a) increased the exercise intensity selected during 1 h of self-paced cycling, which in turn increased by 11.9 ± 20.3% the amount of work performed during the exercise session; (b) increased the total energy expended during exercise by 8.0 ± 16.3%, likely as a result of the increased exercise intensity; (c) did not affect fat and carbohydrate oxidation rates during exercise; (d) did not affect participants' rating of perceived exertion and exercise heart rate; (e) increased the magnitude of several side effects in the 24 hours after ingestion such as nervousness, diuresis and insomnia. Collectively, this information suggests that acute ingestion of 3 mg/kg of caffeine before an exercise session with an individual's freedom to regulate intensity induced participants to select a higher exercise intensity despite a similar rating of fatigue. The increase in exercise intensity with caffeine came with a higher amount of work completed during the exercise session, and a higher amount of energy expended. However, the selection of a higher exercise intensity offset the effect of caffeine on shifting substrate oxidation during aerobic exercise found in investigations with steady-state exercise intensity protocols [6].

The effect of caffeine on fat oxidation has been widely debated because it has been found in some investigations [4, 5, 8–10] but not in others [11, 12]. The differences in the outcomes between these investigations are likely associated with the exercise protocol used to assess caffeine's effect on substrate oxidation. In investigations that used steady-state exercise intensity protocols, caffeine was habitually effective to enhance fat oxidation [6], at expense of lower utilization of carbohydrates [8, 9, 25]. On the other hand, the investigations that employed exercise protocols with free-chosen exercise intensity to assess caffeine's ergogenicity failed to find any effect of caffeine on substrate oxidation [11]. This is because in investigations aimed to assess the ergogenic effect of caffeine, exercise intensity is high (participants complete the trial as fast as they could) and the contribution of fat to energy is low or negligible. This speculation drove the design employed in the current investigation and for this reason, we designed an experiment where participants were told to exercise at moderate intensity to maximize fat oxidation. Even in this scenario of moderate exercise intensity, the data confirms that the benefits of acute caffeine intake on exercise performance and fat oxidation are somewhat incompatible. This is because exercise intensity is the main modulator for the fuels used during aerobic exercise [13] and, in the case of autoregulation of the workload with caffeine, individuals can exercise at a higher intensity for the same level of fatigue, which in turn eliminates the potential effect of caffeine on substrate oxidation (Figure 1).

The incompatibility of both caffeine-induced effects (*i.e.*, ergogenic and substrate oxidation) has a mechanistic support. After oral ingestion of caffeine, the substance is rapidly absorbed and distributed across all body tissues, including the brain [26]. In the brain, caffeine possesses the ability to block adenosine-specific receptors which in turn increases the release of neurotransmitters such as norepinephrine,

dopamine, acetylcholine and serotonin [27]. The blockade of adenosine A1 and A2A receptors is the main mechanism behind caffeine's ergogenicity during exercise [27] and may affect the autoregulation of exercise intensity by enabling a hypoalgesic effect and reduced fatigue, as this is the role of several neurotransmitters stimulated by acute intake [14, 28]. In this context, 3 mg/kg of caffeine are effective to increase VO_{2max} and the wattage obtained during a ramp exercise test to fatigue [29], and to enhance the wattage at the second ventilatory threshold [30], supporting the capacity of individuals to pedal at a higher intensity with caffeine. However, the capacity to exercise at a higher intensity with caffeine may be unfavorable to maximize fat oxidation during exercise. This same scenario was present in the current investigation as participants selected a higher exercise intensity to complete the 1 h of cycling in the caffeine trial (on average, they changed from 105±44 W with placebo to 117±45 W with caffeine) despite they were told that they had to exercise at a moderate intensity to maximize fat oxidation. This produced a VO_2 in the caffeine trial that represented ~57% of VO_{2max} while it was ~51% of VO_{2max} in the placebo trial. The selection of higher intensity with caffeine was present despite participants' self-perceived fatigue being similar to the placebo trial (Figure 1). Interestingly, the results are completely different when using a similar protocol of 1 h of cycling but a with fixed exercise workload at Fatmax through all the trial [5]. In this alternative context, the same dose of caffeine was effective to enhance fat oxidation, reduce carbohydrate oxidation and dampen the sensation of fatigue.

The outcomes of the current investigation and the previous one with fixed exercise workload at Fatmax [5] reveal interesting practical applications for exercise practitioners interested in the use of caffeine supplementation to modify energy expenditure and shift substrate oxidation during exercise. In the case of seeking

increased fat oxidation during exercise, oral caffeine intake should be planned before an exercise session designed with a fixed intensity of moderate level, close to Fatmax [5] or below 60% of VO_{2max} [4], as the effect of caffeine to shift substrate oxidation towards a higher reliance on fat disappears at higher exercise intensities. In the case of pursuing increased energy expenditure during exercise with caffeine, the exercise session should be designed with an auto-selection of exercise intensity, using the rating of perceived exertion as the variable to regulate intensity. In any case, the current investigation and the previous study on this topic [5] reveal that oral caffeine intake increases resting blood pressure and the prevalence and magnitude of several side effects such as nervousness, self-perceived vigor, diuresis and insomnia, independently of the type of exercise used (fixed vs autoselected). Although the cardiovascular effects of caffeine to increase resting blood pressure are eliminated after ~1 week of a chronic intake, the prevalence of the remaining side effects increases over time if caffeine is ingested daily [31]. Therefore, the use of oral caffeine intake to enhance fat oxidation or energy expenditure during exercise should be evaluated in terms of risk/benefits and habituation to this stimulant term should be avoided.

This study presents different limitations that should be discussed: (i) the study participants were healthy and active, and the effect of caffeine intake on fat oxidation should be investigated in other populations such as endurance-trained athletes and obese/overweight individuals; this may be particularly important for endurance-trained athletes as the effect of caffeine to enhance fat oxidation may be lower than in recreationally active individuals [6]; (ii) we obtained only samples of expired air during exercise while blood samples would have been helpful for the analysis of circulating free fatty acids and glycerol as the concentration of these substances are normally increased after caffeine intake [32, 33]; (iii) we investigated the effect of only one dose

of caffeine while the effect of higher doses may have produced different outcomes of the shifting of substrate oxidation [33] (iv) the study included a mixed sample with male and female participants. A sub-analysis of the current results per sex indicated that caffeine increased exercise in a similar magnitude in both men and women, but caffeine did not affect substrate oxidation in men or women. This agrees with recent data that indicate that responses to caffeine in the exercise context are of similar magnitude in men and women [34, 35]. However, further investigations are needed to determine the effect of caffeine on auto-regulated exercise and fat oxidation as the sub-analysis per sex in the current investigation is likely underpowered.

In summary, acute ingestion of 3 mg/kg of caffeine before a 1-h cycling session with an individual's freedom to regulate intensity enabled participants to exercise at a higher exercise intensity despite a similar rating of fatigue. The increase in exercise intensity with caffeine was accompanied by a higher amount of work completed, and a higher amount of energy expended during exercise. However, the potential effect of caffeine on shifting substrate oxidation towards a higher reliance on fat was not present. In the case of seeking increased fat oxidation during exercise, oral caffeine intake should be planned before an exercise session designed with a constant workload of moderate intensity close to Fatmax instead of the use of self-pacing, as caffeine intake before steady-state exercise at Fatmax is effective to enhance fat oxidation [5].

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CONFLICT OF INTEREST

The 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; and no other relationships or activities that could appear to have influenced the submitted work.

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Figure 1. Exercise workload (a), energy expenditure rate (b), fat oxidation rate (c), carbohydrate oxidation rate (d), heart rate (e) and rating of perceived exertion (f) during 1h of cycling at self-paced intensity after the ingestion of 3 mg/kg body mass of caffeine or a placebo.

(*) Significant differences between caffeine and placebo at $P < 0.050$. a.u. = arbitrary units.

TABLES

Table 1. Cardiovascular variables at rest, and work, energy expenditure and substrates oxidized during 1h of cycling at self-paced intensity after the ingestion of 3 mg/kg of caffeine or a placebo.

	Variables (units)	**Placebo**	**Caffeine**	***P* value**
At rest	Heart rate (beats/min)	55±10	53±7	0.172
	Systolic blood pressure (mmHg)	116±13	123±15*	0.004
	Diastolic blood pressure (mmHg)	69±8	74±11*	0.020
	Mean arterial pressure (mmHg)	85±9	90±12*	0.006
During exercise	Total work (kJ)	377±157	422±160*	< 0.001
	Total energy expenditure (kcal)	543±161	587±155*	0.042
	Total fat oxidation (g)	24.7±12.2	23.9±11.6	0.509
	Total carbohydrate oxidation (g)	87.4±22.4	97.8±32.3	0.101
	Sweat rate (L/h)	0.38±0.25	0.48±0.29	0.066

Data is shown as mean±SD for 15 healthy participants. (*) Statistically significant difference from placebo at $P < 0.050$.

Table 2. Ratings of main adverse effects in the 24 hours following the ingestion of 3 mg/kg of caffeine or a placebo.

Variable (units)	Placebo	Caffeine	*P* value
Nervousness (a.u.)	1.3±1.0	3.7±2.3*	0.003
Vigor (a.u.)	1.5±1.6	4.4±2.7*	0.005
Irritability (a.u.)	1.5±1.4	1.6±1.4	0.903
Muscle pain (a.u.)	1.5±1.4	1.3±1.3	0.796
Headache (a.u.)	1.9±2.0	1.5±1.8	0.639
Gastrointestinal distress (a.u.)	1.1±0.5	2.0±1.9	0.126
Diuresis (a.u.)	1.3±0.6	2.9±2.2*	0.009
Insomnia (a.u.)	1.1±0.4	2.8±2.7*	0.035

Data is shown as mean±SD for 15 healthy participants. Each side effect was self-reported by using a 1-10 arbitrary units (a.u.) scale. (*) Statistically significant difference from placebo at $P < 0.050$.

Figure 1

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