Caffeine increases whole-body fat oxidation during 1 h of cycling at Fatmax

Carlos Ruiz-Moreno[1] · Jorge Gutiérrez-Hellín[2] · Francisco J. Amaro-Gahete[1] · Jaime González-García[1] · Verónica Giráldez-Costas[1,3] · Víctor Pérez-García[1] · Juan Del Coso[4]

Abstract

Purpose The ergogenic effect of caffeine on exercise of maximum intensity has been well established. However, there is controversy regarding the effect of caffeine on shifting substrate oxidation at submaximal exercise. The aim of this study was to investigate the effect of acute caffeine ingestion on whole-body substrate oxidation during 1 h of cycling at the intensity that elicits maximal fat oxidation (Fatmax).

Methods In a double-blind, randomized, and counterbalanced experiment, 12 healthy participants (VO_{2max} = 50.7 ± 12.1 mL/kg/min) performed two acute experimental trials after ingesting either caffeine (3 mg/kg) or a placebo (cellulose). The trials consisted of 1 h of continuous cycling at Fatmax. Energy expenditure, fat oxidation rate, and carbohydrate oxidation rate were continuously measured by indirect calorimetry.

Results In comparison to the placebo, caffeine increased the amount of fat oxidized during the trial (19.4 ± 7.7 vs 24.7 ± 9.6 g, respectively; P = 0.04) and decreased the amount of carbohydrate oxidized (94.6 ± 30.9 vs 73.8 ± 32.4 g; P = 0.01) and the mean self-perception of fatigue (Borg scale= 11 ± 2 vs 10 ± 2 arbitrary units; P = 0.05). In contrast, caffeine did not modify total energy expenditure (placebo = 543 ± 175; caffeine = 559 ± 170 kcal; P = 0.60) or mean heart rate (125 ± 13 and 127 ± 9 beats/min; P = 0.30) during exercise. Before exercise, caffeine increased systolic and diastolic blood pressure whilst it increased the feelings of nervousness and vigour after exercise (P < 0.05).

Conclusion These results suggest that a moderate dose of caffeine (3 mg/kg) increases the amount of fat oxidized during 1 h of cycling at Fatmax. Thus, caffeine might be used as an effective strategy to enhance body fat utilization during submaximal exercise. The occurrence of several side effects should be taken into account when using caffeine to reduce body fat in populations with hypertension or high sensitivity to caffeine.

Keywords Endurance exercise · Substrate oxidation · Adverse effects · Stimulant · Performance

Introduction

Caffeine (1,3,7 trimethylxanthine) is a natural alkaloid with a potent ergogenic effect on several forms of maximum intensity exercise [1]. To this regard, a myriad of experiments have confirmed that acute caffeine intake, in a dose between 3 and 9 mg per kg of body mass, has the capacity of increasing the work produced during a wide spectrum of exercise and sport situations [2, 3]. The strong evidence supporting the ergogenic effect of caffeine has led the inclusion of this substance in the list of dietary supplements with good-to-strong evidence of achieving benefits to performance of the International Olympic Committee [4]. There is ample consensus to consider the antagonistic role of caffeine on adenosine A_1, A_{2A} and A_{2B} receptors as the main

✉ Juan Del Coso
juan.delcoso@urjc.es

1 Exercise Physiology Laboratory, Camilo José Cela University, Madrid, Spain

2 Faculty of Education, Francisco de Vitoria University, Madrid, Spain

3 Department of Physical Education, Sport and Human Movement, Autonomus University of Madrid, Madrid, Spain

4 Centre for Sport Studies, Rey Juan Carlos University, Camino del Molino, s/n, 28943 Fuenlabrada, Spain

mechanism behind its ergogenic effects [5], although other alternative mechanism has been proposed such as increased muscle oxygen saturation, potassium ion attenuation in the interstitium and calcium iron release from the sarcoplasmic reticulum [6–8]. The capacity of caffeine to enhance fat oxidation while reducing carbohydrate utilization during endurance exercise has also been proposed as an beneficial effect of acute caffeine intake [9], although its impact to enhance exercise of maximal exercise intensity may be limited to endurance exercise situations in which glycogen sparing is associated to enhanced performance. Nevertheless, the potential capacity of caffeine to increase fat oxidation during exercise of submaximal exercise intensity may be associated to other benefits beyond exercise and sport performance, such as a more effective body fat reduction in individuals involved in exercise programmes to promote weight loss.

The capacity of caffeine to shifting substrate oxidation during endurance exercise of submaximal intensity is perhaps the most debated effect of caffeine despite it was the main finding of one of the pivotal investigations on this topic [10]. Since then, contradictory outcomes have been published in favour or against caffeine's effectiveness to increase fat oxidation during exercise, perhaps because some investigations used protocols to measure both ergogenic and substrate oxidation effects of caffeine at the same time. To this regard, the increase in exercise performance induced by caffeine may counteract the effect of caffeine on substrate oxidation, as exercise intensity is the main modulator for the use of fat and carbohydrate during endurance exercise [11].

On one hand, several investigations have found that acute caffeine intake (3–9 mg/kg) was did not modify fat oxidation during exercise [12–16]. In comparison, other investigations have found that caffeine increased fat oxidation rate during exercise of moderate intensity when using similar dosages and exercise protocols [17–22]. The divergence of these investigations' outcomes might be associated with the experimental protocols used to test the effect of caffeine on substrate oxidation. Investigations of the former [12–16] aimed to assess the ergogenic effect of caffeine and thus, investigators used time trials with freely-chosen wattage or fixed wattage at > 70% of participants' maximal oxygen uptake (VO_{2max}). In the latter investigations [17–22], the main objective was to assess the effect of caffeine on fuel utilization during exercise of submaximal intensity; therefore it were used exercise protocols with lower exercise intensities and fixed wattage. Thus, it seems necessary to select exercise of submaximal intensity, in addition to exercise protocols with fixed wattage, to properly study the effect of caffeine on substrate utilization.

From a practical perspective, the most effective manner of maximizing fat oxidation during submaximal exercise is by selecting the exercise intensity that produces maximum rates of fat oxidation (i.e., Fatmax; [23]) and maintaining this selected intensity over time [24]. Typically, fat oxidation becomes maximal at moderate exercise intensity, where most of the energy demands can be satisfactorily met by fat oxidation [9]. However, no investigation has studied the effect of acute caffeine intake on substrate oxidation during continuous exercise at the intensity that elicits peak fat oxidation. Thus, the aim of this study was to investigate the effect of caffeine on fat and carbohydrate oxidation during 1 h of cycling at Fatmax.

Methods

Participants

Twelve young and healthy participants (8 men and 4 women) volunteered to participate in the study (age = 29 ± 6 years, body mass = 70.2 ± 9.2 kg, height = 1.75 ± 0.08 m, maximal oxygen uptake [VO_{2max}] = 50.7 ± 12.1 mL/kg/min). A pre-experimental sample size calculation indicated that at least 11 participants were needed to obtain statistically significant differences when fat oxidation rate was 0.12 g/min higher with caffeine than with the placebo. The sample size calculation was based on the effect of acute caffeine intake when cycling at Fatmax [9], and it was designed to obtain a statistical power of 80% with type I error set at 5%. Participants fulfilled the following inclusion criteria: (a) age between 18 and 40 years; (b) consistent recreational exercise training of ~ 1 h/day, at least 4 days/week for the previous two months; (c) low habitual caffeine consumption (i.e., < 50 mg of caffeine per day in the previous 3 months), as defined by Filip et al. [25]; (d) in women, regular duration of their menstrual cycle for the previous 4 months. Participants were excluded if they reported (a) any type of lower limb injury within the previous two months; (b) a positive smoking status; (c) medication or dietary supplement usage within the previous month; (d) a previous history of cardiopulmonary diseases, (e) oral contraceptive use; (f) allergy to caffeine; (g) in women, any type of menstrual disorders. Women performed all experimental trials during their luteal phase to avoid the influence of the menstrual cycle on the results of the investigation despite evidence suggests that fuel oxidation during submaximal intensity exercise [26, 27] and the response to acute caffeine intake during exercise are similar across the menstrual cycle [28, 29]. Before enrolment in the study, all participants were fully informed of the experimental procedures and risks and signed an informed written consent. The study was approved by the Camilo José Cela University Research Ethics Committee and was conducted in accordance with the last version of the Declaration of Helsinki.

Experimental design

A double-blind, placebo-controlled, randomized, and counterbalanced experimental design was used in this investigation. Each participant took part in two identical experimental trials separated by at least three days. In these trials, participants either ingested 3 mg of caffeine per kg of body mass (Bulk Powders, United Kingdom) or 3 mg/kg of placebo (cellulose, Guinama, Spain). A dose of 3 mg/kg of caffeine was selected as this dosage has been found effective to increase fat oxidation at Fatmax [9]. The treatments were ingested in identical, unidentifiable capsules 60 min before the onset of exercise. An alphanumeric code was assigned to each trial by a researcher independent of the study in order to double-blind the participants and investigators to the tested substances. This alphanumeric code was only unveiled after the trials had been analysed. Each trial consisted of 1 h of continuous cycling at Fatmax while energy expenditure and substrate oxidation of carbohydrates and fat were continuously measured by indirect calorimetry. One hour of continuous cycling at Fatmax was selected as the exercise protocol to assess the effect of caffeine on fat oxidation during exercise because this same protocol has been effective at detecting differences in fat oxidation after the ingestion of other phytochemicals [24]. The trials were performed in a laboratory room with controlled ambient temperature (20.3 ± 0.4 °C) and relative humidity (31 ± 11%).

Pre experimental trial

Participants performed two pre-experimental trials: one maximal cycling test to obtain Fatmax and VO_{2max} during a cycling test of increasing intensity and one familiariza- tion trial. One week before the start of the experiment, participants underwent a maximal cycling test until volitional fatigue. This test was preceded by a standardized warm-up (i.e. 10 min at 50 W for men, and 30 W for woman) on a cyclergometer (SNT medical, Cardgirus, Spain). After the warm-up, the workload was increased by 25 W for men and 15 W for women every 3 min until the respiratory exchange ratio reached 1.0. Thereafter, incremental loads were produced every minute until the participant reached volitional exhaustion. Participants were instructed to maintain a cycle cadence of 70–90 rpm during the whole test. The maximal exercise test finished when participants were unable to maintain a cycle cadence > 50 rpm or when they stopped pedalling due to fatigue. During the maximal exercise test, oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were measured breath by breath by a gas analyser (Metalyzer 3B, Cortex, Germany) and substrate oxidation rates were calculated at each stage with stoichiometric calculations. In this test, the intensity that produced the maximal rate of fat oxidation was registered and used for the experimental trials (Fatmax; mean ±standard deviation was 108 ± 50 W). Three days before the first experimental trial, participants underwent a familiarization trial that replicated the protocols of the experimental trials, as explained below.

Standardizations

Once the participants fulfilled the inclusion/exclusion criteria, they were informed about the necessity of refraining from all sources of caffeine (coffee, tea, chocolate, energy drinks, etc.) until the experiment was completed. Participants were also encouraged to maintain their training routines and a stable state of physical fitness during the experiment. The day before each trial, participants performed light, standardised training and a self-selected precompetitive diet/fluid routine was kept. Fluid and diet guidelines were given to assure carbohydrate availability [30] and euhydration [31] in all experimental trials. Subjects were also required to refrain from consuming alcohol and to maintain a sleeping pattern with at least 8 h of sleep the day before each trial. These standardisations were written in a personal journal and were repeated thoroughly the day before the second trial. Data on diet and exercise were analysed afterwards to ensure that participants fulfilled all the recommendations given and euhydration was certified by urine specific gravity < 1.020 before each testing (MASTER-SUR/Nα, Atago, Japan). In women, regularity and duration of the menstrual cycle were monitored in each participant for 4 months through a free mobile application (Mycalendar®,Period-tracker, US).

Experimental trials

On the day of the experimental trials, participants arrived at the laboratory in the morning (09.00 am) in a fasted state (at least 8 h after their last meal). Upon arrival, the capsule with the experimental treatment (caffeine or placebo) was provided and ingested by the participant with 150 mL of water. Then, participants rested supine for 60 min to allow for substance absorption. In the last 5-min of the resting period, participants' blood pressure (M6 Comfort, Omron, Japan; by triplicate) and heart rate (Wearlink + V800, Polar, Finland) were recorded. An average of three blood pressure measurements, performed with 1 min between measurements, was used for analysis. Thereafter, participants underwent a standardized 10-min warm-up with increasing intensity until they reached their individual Fatmax (equivalent to 52.1 ± 9.8% of their VO_{2max}). The cyclergometer was set to maintain this exercise intensity and participants pedalled at this intensity for 1 h. The position of the saddle and handlebar in the cyclergometer, clothing used, and cycling cadence were meticulously replicated in both trails. VO_2 and VCO_2 data were obtained breath by breath during the whole trial with the gas analyser used for the VO_{2max} test. VO_2

and VCO_2 data within the last 60 s of each 5-min period was used as a representative value. During the last 60 s of each 5-min period, participants were instructed to maintain a stable position and cadence. The rates of energy expenditure and substrate oxidation were calculated from VO_2 and VCO_2 using the non-protein respiratory quotient [32]. Data on VO_2 and VCO_2 for the whole trial (i.e., all the breaths included in the 1-h cycling trial) were used for the calculation of the total amount of energy expended and total amounts of fat and carbohydrate oxidized. During exercise, heart rate and participants' rating of perceived exertion (6–20 arbitrary units (a.u.) scale [33]) were recorded at 5-min intervals.

After the end of the trials, participants completed an ad hoc questionnaire regarding common side effects after acute caffeine intake. This questionnaire included a 1- to 10-point scale to assess each item. Participants were previously informed that one point meant a minimal amount of that item and 10 points meant a maximal amount. Participants were provided with an online version of this questionnaire via WhatsApp, and the questionnaire contained two phases. In the first phase, participants had to rate their feelings of nervousness, vigour, irritability, gastrointestinal problems, muscular pain, headache, and diuresis and they completed these questionnaire 12 h after the end of exercise to assess the intensity of the side effects induced by acute caffeine intake in the day of the experiment. In the second phases of the questionnaire, participants rated their level of insomnia during the night after the experimental trial. This survey was completed in the following morning of the experiment once participants had completed their night sleep. This two-phases survey has been effectively used to assess side effects resulting from acute caffeine ingestion in individuals performing several exercise situations [34, 35].

Statistical analysis

The study data were blindly introduced into the statistical package SPSS (SPSS, v. 22.0, IBM SPSS Statistics, IBM Corporation) and subsequently analysed. The Shapiro–Wilk test was used to confirm the normality of the quantitative variables and, consequently, parametric statistics test were used to determine differences among trials. A two-way analysis of variance (ANOVA) (substance ×time) was performed to analyse the main effect of caffeine on all the variables under investigation. After a significant F test (Greenhouse–Geisser correction for the assumption of sphericity) was completed for the main effect of caffeine, differences in the caffeine-placebo comparisons at each 5-min measurement were identified by LSD post-hoc tests. Paired *t*-tests were used to detect differences in the caffeine-placebo comparison in resting heart rate, resting blood pressure, overall values of energy expenditure, fat and carbohydrate oxidation during exercise, and in side-effect ratings post-exercise. In all statistical tests, a significance level of $P < 0.05$ was set. The data are presented as mean ± standard deviation. The effect size (± 95% confidence intervals (CI)) was calculated in all pairwise comparisons [36]. The smallest significant effect threshold was set as 0.2, and a qualitative descrip- tor was included to represent the likelihood of exceeding this threshold. Ranges of likelihood < 1% indicated almost certainly no chances of change; 1% to 5%, very unlikely; 5% to 25%, unlikely; 25% to 75%, possible; 75% to 95%, likely; 95% to 99%, very likely; > 99%, most likely. Differences were rated as unclear when likelihood exceeded > 5% in both positive/negative directions [37].

Results

Before exercise, the acute ingestion of caffeine increased systolic, diastolic, and mean arterial blood pressure in comparison to the ingestion of the placebo (Table 1; $P < 0.05$). However, caffeine did not modify resting heart rate ($P = 0.62$).

During exercise, there was a significant effect of caffeine on fat oxidation rate ($P = 0.02$). The post-hoc analysis revealed that the rate of fat oxidation was higher with caffeine than with the placebo in all pairwise comparisons during the 1-h cycling trial (all $P < 0.05$, Fig. 1a). Moreover, there was a main effect of caffeine on carbohydrate oxidation rate ($P = 0.02$) while the post-hoc analysis revealed that caffeine decreased carbohydrate oxidation rate from min- 10 to min-60 (all $P < 0.05$, Fig. 1b). However, there was no main effect of caffeine on energy expenditure ($P = 0.60$; Fig. 1c). As a whole, caffeine increased the total amount of fat oxidized during the trial when compared to the placebo (Table 1; $P = 0.04$) while reducing the amount of carbohydrates utilized ($P < 0.01$). The ingestion of caffeine did not modify the amount of energy expended during the trial ($P < 0.71$).

During exercise, there was a main effect of caffeine on the rating of perceived exertion ($P = 0.05$) with the post-hoc analysis demonstrating that the rating of perceived exertion was lower with caffeine than with the placebo from min-45 to min-60 (all $P < 0.05$, Fig. 2a). In addition, there was a main effect of caffeine on VO_2 ($P = 0.03$) with the post-hoc analysis indicating that VO_2 was higher with caffeine than with the placebo at several time-points during exercise (all $P < 0.05$, Fig. 2b). However, there was no main effect of caffeine on heart rate ($P = 0.20$; Fig. 2c).

After exercise, participants reported higher ratings of nervousness and vigorousness in the caffeine trial vs the placebo trial (Table 2; $P < 0.05$). Although there were no statistical differences in the remaining side effects, the effect sizes of acute caffeine on gastrointestinal distress, diuresis, and insomnia were > 1.0.

Table 1 Cardiovascular variables at rest, and energy expenditure and substrates oxidized during 1 h of cycling at the intensity that elicits maximal fat oxidation (Fatmax) after the ingestion of caffeine or a placebo

	Variables (units)	Placebo	Caffeine	Effect Size (95% CI)	Qualitative inference	*P* value
Resting	Heart rate (beats/min)	54 ± 9	55 ± 9	0.2 (− 0.3/0.6)	Unclear	0.62
	Systolic blood pressure (mmHg)	116 ± 13	124 ± 20	0.5 (0.2/0.8)	Very likely	0.01
	Diastolic blood pressure (mmHg)	71 ± 10	74 ± 11	0.3 (0.1/0.6)	Likely	0.02
	Mean blood pressure (mmHg)	86 ± 10	91 ± 13	0.5 (0.2/0.7)	Very likely	0.01
Exercise	Total fat oxidation (g)	19.4 ± 7.7	24.7 ± 9.6	0.5 (0.1/0.9)	Likely	0.04
	Total carbohydrate oxidation (g)	94.6 ± 30.9	73.8 ± 32.4	– 0.8 (– 1.4/– 0.3)	Very likely	< 0.01
	Total energy expenditure (kcal)	543 ± 175	559 ± 170	0.0 (– 0.2/0.1)	Very likely	0.71
	Mean heart rate (beats/min)	125 ± 13	127 ± 9	0.2 (– 0.1/0.4)	Possible	0.30
	Mean respiratory exchange ratio	0.89 ± 0.04	0.85 ± 0.05	– 1.1 (– 1.9/– 0.4)	Very likely	0.04

Data is shown as mean ±SD for 12 healthy participants

CI confidence interval

Discussion

The main outcomes of this research evidenced the potential effects of caffeine, when ingested on a moderate dose (3 mg/kg) before exercise, on shifting substrate oxidation during exercise towards a higher reliance of fat as a fuel source. The effect of caffeine was evident during all time comparisons, suggesting that caffeine's effectiveness at enhancing fat oxidation was present from the beginning to the end of the exercise trial. As a result, caffeine increased the amount of fat oxidized during 1 h of cycling at Fatmax by 27.0%. Interestingly, the higher fat oxidation associated with caffeine was accompanied by an associated caffeine-induced reduction in total carbohydrate oxidation (-22.0%), with no differences in energy expenditure. Despite caffeine's potential benefit in shifting substrate oxidation during exercise, the intake of caffeine was associated with two drawbacks: the increase in systolic and diastolic blood pressure before exercise and the increase in the self-reported ratings of several side-effects in the 24 h after exercise. Together, this information suggests that caffeine might be considered as an effective substance to augment fat oxidation during continuous exercise at submaximal intensity. However, caffeine might not be recommended to individuals with hypertension or excessive sensitivity to caffeine-related side effects.

After acute ingestion, caffeine is rapidly absorbed. As a result of its lipophilic nature, it passes through all biological membranes, including the blood–brain barrier [38]. In this regard, there is ample consensus to consider the antagonistic role of caffeine as the main mechanism behind its ergogenic effects, primarily due to its interactions with adenosine A_1, A_{2A} and A_{2B} receptors in the central nervous system [5]. Caffeine can also exert effects during submaximal exercise via increased release of epinephrine, mobilization of fatty acids from adipose tissue, [12, 13] and increased muscle oxygen saturation [8]. These effects might allow for a higher availability of fatty acids and oxygen to allow for a higher reliance of fat oxidation as a fuel. Yet, it also seems that caffeine's effect on fat oxidation might be at the mercy of exercise intensity.

Exercise intensity is the main determinant for the body's selection between fat and carbohydrates as energy sources during exercise. It is also worth noting that exercise duration, environmental conditions, training status, and pre-exercise diet can also influence fat oxidation levels during exercise [39]. Overall, fat oxidation rate increases with exercise intensity until moderate exercise intensity. It then abruptly decreases with further increases in exercise intensity, once the maximal fat oxidation rate is obtained to produce a U-shaped curve that associates exercise inten- sity and fat oxidation rate [23]. Previous investigations have determined that the maximal rate of fat oxidation is obtained around 40 to 75% of VO_{2max}, although differences might be found between trained and untrained individuals [23, 40]. In the investigations where caffeine did not change substrate oxidation during exercise, the exercise intensity was > 70% VO_{2max} [12–14] or it was freely chosen during a time trial [15, 16]. In contrast, the investigations where caffeine increased fat oxidation during exercise used exercise intensities between 50 and 65% VO_{2max} [17–20], or equivalent to Fatmax [21], or equivalent to the participants' maximal lactate steady state [22]. In this investigation, participants cycled at Fatmax, which represented 52.1 ± 9.8% of the participants' VO_{2max}. By pooling all these outcomes, it can be inferred that caffeine may enhance fat oxidation during submaximal endurance exercise with intensities close to or below Fatmax. In contrast, in intensities above Fatmax, caffeine may be ineffective in changing substrate oxidation. This inference is supported by a recent investigation that tested the effect of acute caffeine intake on fat oxidation during a ramp exercise test [9]. In that investigation, caffeine increased fat oxidation during exercise at 30-to-70%

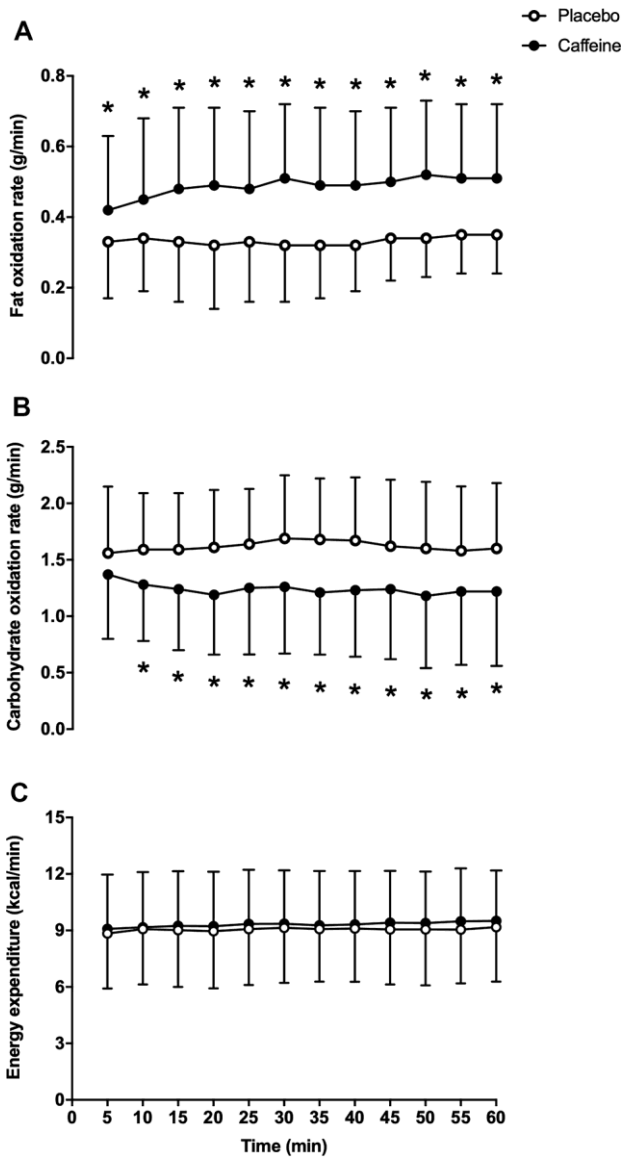

Fig. 1 Fat oxidation rate (**a**), carbohydrate oxidation rate (**b**), and energy expenditure rate (**c**) during 1 h of cycling at the intensity that elicits maximal fat oxidation (Fatmax) after the ingestion of caffeine or the placebo. (*) Significant differences between caffeine and placebo at $P < 0.05$

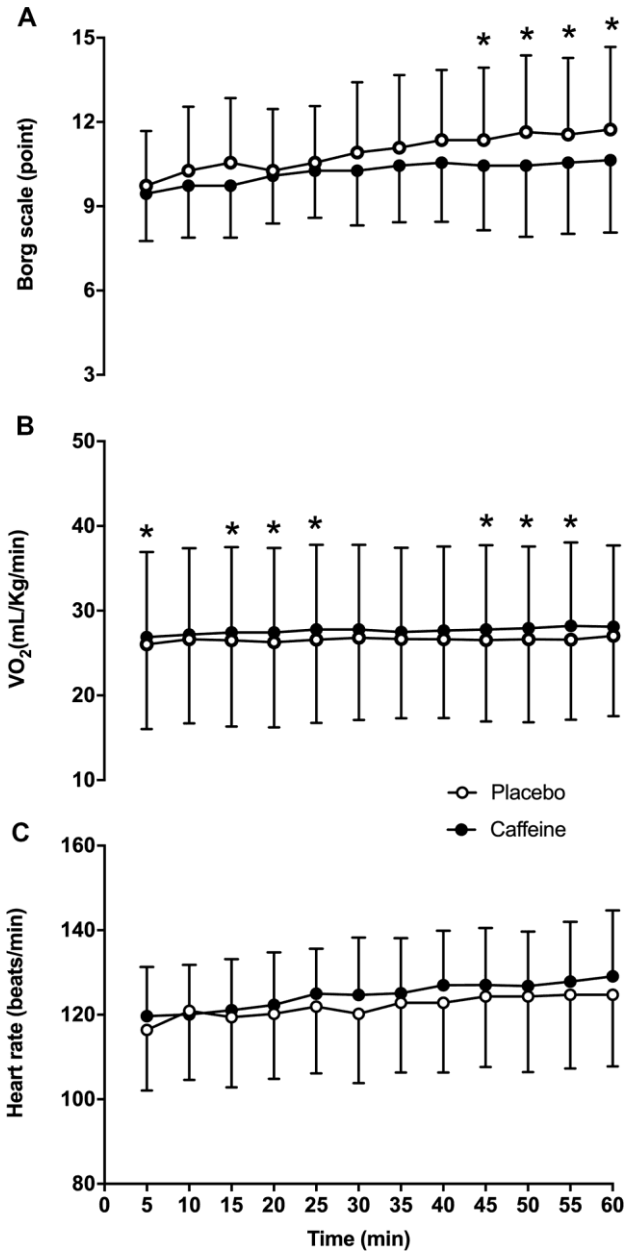

Fig. 2 Self-reported rating of perceived exertion (**a**), VO_2 (**b**), and heart rate (**c**) during 1 h of cycling at the intensity that elicits maxi- mal fat oxidation (Fatmax) after the ingestion of caffeine or placebo. (*) Significant differences between caffeine and placebo at $P < 0.05$. VO_2: oxygen uptake

VO_{2max}, but was unable to affect substrate oxidation when exercise intensity was ≥ 80% VO_{2max}. These outcomes suggest that caffeine may increase the reliance on fat as a fuel at Fatmax. At higher exercise intensities, caffeine will exert an ergogenic effect [10, 12, 15], which is independent of changes on substrate oxidation.

Another interesting finding of this investigation is the effect of caffeine on reducing the ratings of perceived exertion during exercise, particularly at the end of exercise. Due to the blockage of adenosine receptors, caffeine indi- rectly augments the release of norepinephrine, dopamine, acetylcholine, and serotonin [41]. The release of these neurotransmitter produces a scenario for dampened pain during exercise, blunting perceived exertion [42]. A meta-analysis associating the effect of caffeine on self-reported fatigue and ergogenicity found that caffeine's effect on reducing the rating of perceived exertion during exercise accounted for 29% of the variance in exercise performance improvement [43]. In the present investigation, exercise performance was not

Table 2 Ratings of main side effects in the 24 h after the ingestion of caffeine or a placebo

Variable (units)	Placebo	Caffeine	Effect size (95%CI)	Qualitative inference	*P* value
Nervousness (a.u.)	1.3 ± 1.2	3.7 ± 2.4	2.1 (1.2/2.9)	Most likely	0.01
Vigour (a.u.)	1.6 ± 1.8	4.7 ± 2.9	1.8 (0.8/2.8)	Very likely	0.04
Irritability (a.u.)	1.2 ± 0.6	1.5 ± 1.5	0.2 (– 0.8/1.2)	Unclear	0.73
Muscle pain (a.u.)	1.6 ± 1.5	1.4 ± 1.4	– 0.1 (– 0.8/0.5)	Unclear	0.79
Headache (a.u.)	2.1 ± 2.2	1.6 ± 2	– 0.2 (– 0.9/0.5)	Unclear	0.66
Gastrointestinal distress (a.u.)	1.1 ± 0.6	2.2 ± 2.0	1.2 (0.2/2.3)	Likely	0.12
Diuresis (a.u.)	1.2 ± 0.6	2.4 ± 2.1	1.2 (0.0/2.3)	Likely	0.16
Insomnia (a.u.)	1.1 ± 0.4	2.8 ± 2.7	1.8 (0.1/3.5)	Likely	0.11

Data is shown as mean±SD for 12 healthy participants. Each side effect was self-reported by using 1–10 arbitrary units (a.u.) scale

CI confidence interval

investigated as exercise intensity and duration were kept constant in both experimental trials. However, the reduced exertion found after the ingestion of caffeine despite maintaining the same exercise intensity might have also helped to enhance fat oxidation during exercise as participants reported that they felt the workload lighter with caffeine than with the placebo. Overall, the ingestion of caffeine before an exercise trial of submaximal exercise induces the release of neurotransmitters and catecholamines that may (1) produce a higher bioavailability of fatty acids and (2) lower exertion that may ultimately result in a higher reliance on fat as an energy source for muscles in contraction.

The acute ingestion of caffeine was accompanied by several pre-exercise and post-exercise adverse effects. Before exercise, caffeine increased systolic and diastolic pressure. Although the caffeine-induced change in blood pressure had little clinical impact on this sample of healthy and active individuals, this might be a negative effect for those with elevated blood pressures seeking to improve fat oxidation with caffeine. Interestingly, the daily intake of 3 mg/kg of caffeine produces that the caffeine-induced effect on blood pressure disappeared after 8 days of continuous ingestion [44], suggesting that there is tolerance to the cardiovascular effect of caffeine at rest. After exercise, caffeine increased self-reported ratings of nervousness and vigour. There were also self-reported drawbacks such as gastrointestinal distress, diuresis, and insomnia. These side effects have been found in similar cases of athletes seeking to enhance physical performance by ingesting the same dosage of caffeine [34, 35, 45]. Contrary to the effect of caffeine on blood pressure, caffeine's side effects such as increased nervousness and vigour, irritability, insomnia, and diuresis increased with chronic ingestion (i.e., up to 20 days; [44]). In light of these results, caffeine should only be recommended for individuals seeking to augment fat oxidation during exercise and who do not have any previous history of cardiovascular problems or high caffeine sensitivity. In addition, due to the tolerance to some of the most important ergogenic effects of caffeine [46] and caffeine's selective efficacy in enhancing fat oxidation at submaximal exercise, caffeine intake should only be recommended for exercise sessions that entail continuous exercise at Fatmax.

There are limitations associated with this investigation that should be considered to improve the results' applicability. First, we did not obtain blood samples nor tissue samples. Thus, we were unable to separate the effect induced by caffeine intake on adipose tissue / intramuscular triacylglycerols oxidation. We are also unable to establish the caffeine's ability to deliver released fatty acids to active skeletal muscle. Second, we only used one dose of caffeine (3 mg/kg) and tested its effect on substrate oxidation at one exercise intensity (Fatmax). Further investigations should be aimed at determining if there is a dose–response of caffeine on shifting fat oxidation. It is also necessary to determine the effect of acute caffeine intake on intensities below Fatmax. Finally, we used a sample of both men and women with low habituation to caffeine. An analysis of the subsamples of men and women participants confirmed that the effect of caffeine to shifting substrate oxidation was present in both groups. However, future investigation should determine whether the effect of acute caffeine intake to enhance fat oxidation during exercise of submaximal intensity is similar in both sexes and the time course of tolerance to the benefit of caffeine in enhancing fat oxidation during exercise.

In summary, the acute ingestion of caffeine enhanced fat oxidation during 1 h of cycling at Fatmax at the expense of reduced carbohydrate oxidation. The effect of caffeine on fat oxidation was present during the entire trial and produced a meaningful increment of 27.0% in the amount of fat oxidized during the trial. Caffeine produced a slightly higher blood pressure before exercise and a higher rating of several minor side effects after exercise. Although the clinical relevance of these drawbacks was inconsequential in the sample of healthy individuals employed in this investigation, the occurrence of these side effects should be taken into account when using this stimulant to reduce body fat in populations

with hypertension or high sensitivity to caffeine. Lastly, it is likely that the effect of caffeine on fat oxidation is only present when the intensity of exercise is close to or below Fatmax. This is because higher exercise intensities might dampen caffeine's efficacy to shift substrate oxidation since cellular mechanisms can favour the use of carbohydrates instead of fat.

Acknowledgements The authors wish to thank the subjects for their invaluable contribution to the study.

Funding This investigation did not receive any funding.

Compliance with ethical standards

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.

References

1. Goldstein ER, Ziegenfuss T, Kalman D et al (2010) International society of sports nutrition position stand: Caffeine and performance. J Int Soc Sports Nutr 7:5
2. Salinero J, Lara B, Del Coso J (2019) Effects of acute ingestion of caffeine on team sports performance: a systematic review and meta-analysis. Res Sport Med 27:238–256. https://doi.org/10.1080/15438627.2018.1552146
3. Grgic J, Grgic I, Pickering C et al (2019) Wake up and smell the coffee: Caffeine supplementation and exercise performance - An umbrella review of 21 published meta-analyses. Br J Sports Med 54:681–688. https://doi.org/10.1136/bjsports-2018-100278
4. Maughan RJ, Burke LM, Dvorak J et al (2018) IOC consensus statement: dietary supplements and the high-performance athlete. Br J Sports Med 52:439–455. https://doi.org/10.1136/bjsports-2018-099027
5. Fredholm BB, Yang J, Wang Y (2017) Low, but not high, dose caffeine is a readily available probe for adenosine actions. Mol Aspects Med 55:20–25
6. Graham TE (2001) Caffeine and exercise metabolism, endurance and performance. Sport Med 31:785–807
7. Davis JK, Green JM (2009) Caffeine and anaerobic performance: Ergogenic value and mechanisms of action. Sport Med 39:813–832
8. Ruíz-Moreno C, Lara B, Brito de Souza D et al (2020) Acute caffeine intake increases muscle oxygen saturation during a maximal incremental exercise test. Br J Clin Pharmacol 86:861–867. https://doi.org/10.1111/bcp.14189
9. Gutiérrez-Hellín J, Del Coso J (2018) Effects of p-Synephrine and caffeine ingestion on substrate oxidation during exercise. Med Sci Sport Exerc 50:1899–1906. https://doi.org/10.1249/MSS.0000000000001653
10. Costill DL, Dalsky GP, Fink WJ (1978) Effects of caffeine ingestion on metabolism and exercise performance. Med Sci Sports 10:155–158
11. Venables MC, Achten J, Jeukendrup AE (2005) Determinants of fat oxidation during exercise in healthy men and women: A cross-sectional study. J Appl Physiol. https://doi.org/10.1152/japplphysiol.00662.2003
12. Graham TE, Helge JW, MacLean DA et al (2000) Caffeine ingestion does not alter carbohydrate or fat metabolism in human skeletal muscle during exercise. J Physiol 529:837–847. https://doi.org/10.1111/j.1469-7793.2000.00837.x
13. Graham TE, Battram DS, Dela F et al (2008) Does caffeine alter muscle carbohydrate and fat metabolism during exercise? Appl Physiol Nutr Metab Physiol Appl Nutr Metab 33:1311–1318. https://doi.org/10.1139/H08-129
14. Graham TE, Spriet LL (1995) Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. J Appl Physiol 78:867–874. https://doi.org/10.1152/jappl.1995.78.3.867
15. Beaumont RE, James LJ (2017) Effect of a moderate caffeine dose on endurance cycle performance and thermoregulation during prolonged exercise in the heat. J Sci Med Sport 20:1024–1028. https://doi.org/10.1016/j.jsams.2017.03.017
16. Hodgson AB, Randell RK, Jeukendrup AE (2013) The metabolic and performance effects of caffeine compared to coffee during endurance exercise. PLoS ONE 8:e59561. https://doi.org/10.1371/journal.pone.0059561
17. Anderson DE, Hickey MS (1994) Effects of caffeine on the metabolic and catecholamine responses to exercise in 5 and 28 degrees C. Med Sci Sports Exerc 26:453–458
18. Ryu S, Choi SK, Joung SS et al (2001) Caffeine as a lipolytic food component increases endurance performance in rats and athletes. J Nutr Sci Vitaminol (Tokyo) 47:139–146. https://doi.org/10.3177/jnsv.47.139
19. Donelly K, McNaughton L (1992) The effects of two levels of caffeine ingestion on excess postexercise oxygen consumption in untrained women. Eur J Appl Physiol Occup Physiol 65:459–463. https://doi.org/10.1007/BF00243514
20. Schubert MM, Sabapathy S, Leveritt M, Desbrow B (2014) Acute exercise and hormones related to appetite regulation: a meta-analysis. Sports Med 44:387–403. https://doi.org/10.1007/s40279-013-0120-3
21. Alkhatib A, Seijo M, Larumbe E, Naclerio F (2015) Acute effectiveness of a "fat-loss" product on substrate utilization, perception of hunger, mood state and rate of perceived exertion at rest and during exercise. J Int Soc Sports Nutr 12:44. https://doi.org/10.1186/s12970-015-0105-8
22. Cruz R, de Aguiar R, Turnes T et al (2015) Caffeine affects time to exhaustion and substrate oxidation during cycling at maximal lactate steady state. Nutrients 7:5254–5264. https://doi.org/10.3390/nu7075219
23. Maunder E, Plews DJ, Kilding AE (2018) Contextualising maximal fat oxidation during exercise: Determinants and normative values. Front Physiol 23:599. https://doi.org/10.3389/fphys.2018.00599
24. Gutiérrez-Hellín J, Ruiz-Moreno C, Del Coso J (2019) Acute p-synephrine ingestion increases whole-body fat oxidation dur- ing 1-h of cycling at Fatmax. Eur J Nutr. https://doi.org/10.1007/s00394-019-02101-6
25. Filip A, Wilk M, Krzysztofik M, Del Coso J (2020) Inconsistency in the ergogenic effect of caffeine in athletes who regularly consume caffeine: Is it due to the disparity in the criteria that defines habitual caffeine intake? Nutrients. https://doi.org/10.3390/nu12041087
26. Frandsen J, Pistoljevic N, Quesada JP et al (2020) Menstrual cycle phase does not affect whole body peak fat oxidation rate during a graded exercise test. J Appl Physiol 128:681–687. https://doi.org/10.1152/japplphysiol.00774.2019
27. Horton TJ, Miller EK, Glueck D, Tench K (2002) No effect of menstrual cycle phase on glucose kinetics and fuel oxidation during moderate-intensity exercise. Am J Physiol - Endocrinol Metab. https://doi.org/10.1152/ajpendo.00238.2001
28. Lara B, Gutiérrez-Hellín J, García-Bataller A et al (2019) Ergogenic effects of caffeine on peak aerobic cycling power during the

menstrual cycle. Eur J Nutr. https://doi.org/10.1007/s00394-019-02100-7
29. Lara B, Gutiérrez Hellín J, Ruíz-Moreno C et al (2019) Acute caffeine intake increases performance in the 15-s Wingate test during the menstrual cycle. Br J Clin Pharmacol. https://doi.org/10.1111/bcp.14175
30. Burke LM, Hawley JA, Wong SHS, Jeukendrup AE (2011) Carbohydrates for training and competition. J Sports Sci 29:S17–S27. https://doi.org/10.1080/02640414.2011.585473
31. Sawka MN, Burke LM, Eichner ER et al (2007) Exercise and fluid replacement. Med Sci Sports Exerc 39:377–390
32. Brouwer E (1957) On simple formulae for calculating the heat expenditure and the quantities of carbohydrate and fat oxidized in metabolism of men and animals, from gaseous exchange (Oxygen intake and carbonic acid output) and urine-N. Acta Physiol Pharmacol Neerl 6:795–802
33. Borg GAV (1954) Psychophysical bases of perceived exertion. Plast Reconstr Surg 14:377–381. https://doi.org/10.1249/00005768-198205000-00012
34. Salinero JJ, Lara B, Abian-Vicen J et al (2014) The use of energy drinks in sport: Perceived ergogenicity and side effects in male and female athletes. Br J Nutr 112:1494–1502. https://doi.org/10.1017/S0007114514002189
35. Lara B, Ruiz-Vicente D, Areces F et al (2015) Acute consumption of a caffeinated energy drink enhances aspects of performance in sprint swimmers. Br J Nutr 114:908–914. https://doi.org/10.1017/S0007114515002573
36. Hopkins WG, Marshall SW, Batterham AM, Hanin J (2009) Progressive statistics for studies in sports medicine and exercise science. Med Sci Sports Exerc 41:3–13. https://doi.org/10.1249/MSS.0b013e31818cb278
37. Hopkins WG (2020) A Spreadsheet for deriving a confidence interval, mechanistic inference and clinical inference from a *p* value. https://www.sportsci.org/2007/wghinf.htm. Accessed 20 Jun 2020
38. McCall AL, Millington WR, Wurtman RJ (1982) Blood-brain barrier transport of caffeine: Dose-related restriction of adenine transport. Life Sci 31:2709–2715. https://doi.org/10.1016/0024-3205(82)90715-9
39. Purdom T, Kravitz L, Dokladny K, Mermier C (2018) Understanding the factors that effect maximal fat oxidation. J Int Soc Sports Nutr 15:3. https://doi.org/10.1186/s12970-018-0207-1
40. Randell RK, Rollo I, Roberts TJ et al (2017) Maximal fat oxidation rates in an athletic population. Med Sci Sports Exerc 49:133–140. https://doi.org/10.1249/MSS.0000000000001084
41. Daly J, Shi D (1994) The role of adenosine receptors in the central action of caffeine. Pharmacopsychoecologia 7:201–213. https://doi.org/10.1201/9781439822470.ch1
42. Killen LG, Green JM, O'Neal EK et al (2013) Effects of caffeine on session ratings of perceived exertion. Eur J Appl Physiol 113:721–727. https://doi.org/10.1007/s00421-012-2480-z
43. Doherty M, Smith PM (2005) Effects of caffeine ingestion on rating of perceived exertion during and after exercise: a meta-analysis. Scand J Med Sci Sports 15:69–78. https://doi.org/10.1111/j.1600-0838.2005.00445.x
44. Ruiz-Moreno C, Lara B, Salinero JJ et al (2020) Time course of tolerance to adverse effects associated with the ingestion of a moderate dose of caffeine. Eur J Nutr. https://doi.org/10.1007/s00394-019-02167-2
45. Lara B, Gonzalez-Millán C, Salinero JJ et al (2014) Caffeine-containing energy drink improves physical performance in female soccer players. Amino Acids 46:1385–1392. https://doi.org/10.1007/s00726-014-1709-z
46. Lara B, Ruiz-Moreno C, Salinero JJ, Del Coso J (2019) Time course of tolerance to the performance benefits of caffeine. PLoS ONE 14:e0210275. https://doi.org/10.1371/journal.pone.0210275