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EFFECTS OF DIFFERENT DOSES OF CAFFEINE ON COGNITIVE PERFORMANCE IN HEALTHY PHYSICALLY ACTIVE INDIVIDUALS

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Abstract:	Purpose Caffeine is a potent central nervous system stimulant that increases the activity of the prefrontal cortex and can improve cognitive skills. An improvement in these cognitive skills can lead to further benefits in athletic performance; therefore, it is necessary to clarify the dose-response of caffeine on cognitive performance. This study aimed to determine the effects of different doses of caffeine on sport-related cognitive aspects. Methods Twenty-nine healthy physically active young adults were recruited. All participants completed three trials under the following conditions: a) placebo, b) 3 mg/kg, or c) 6 mg/kg body mass of caffeine. In each trial, different cognitive abilities were evaluated with the following battery of tests: reaction time (Dynavision™ D2), anticipation (Bassin Anticipation Timer), sustained attention (Go/No-Go and Eriksen Flanker Test) and memory tests. Moreover, the side effects and the perceived sensation index were recorded 24 h after each test. Results	

	Reaction time only improved following 6 mg/kg of caffeine intake (Physical reaction time: -0.04 s, 95% CI -0.08 to -0.01 s, $P=0.036$, $d=0.5$; Motor reaction time: -0.04 s, 95% CI -0.07 to -0.01 s, $P=0.008$, $d=0.6$) compared to the placebo. Anticipation, sustained attention, and memory were not affected after either caffeine dose intake (all $P>0.05$). In addition, the 6 mg/kg dose of caffeine augmented the occurrence of the side effects of increased activeness ($P=0.046$) and nervousness ($P=0.001$). Conclusion Acute intake of 6 mg/kg caffeine is effective in improving reaction time despite increasing the occurrence of side effects in healthy physically active young adults.
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TITLE: EFFECTS OF DIFFERENT DOSES OF CAFFEINE ON COGNITIVE PERFORMANCE IN HEALTHY PHYSICALLY ACTIVE INDIVIDUALS

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ABSTRACT

Purpose Caffeine is a potent central nervous system stimulant that increases the activity of the prefrontal cortex and can improve various cognitive skills. An improvement in these cognitive skills can lead to further benefits in athletic performance. Therefore, it is necessary to clarify the dose-response of caffeine on cognitive performance. This study aimed to determine the effects of different doses of caffeine on sport-related cognitive aspects.

Methods Twenty-nine healthy physically active young adults were recruited. All participants completed three trials under the following conditions: a) placebo, b) 3 mg/kg, or c) 6 mg/kg body mass of caffeine. In each trial, different cognitive abilities were evaluated with the following battery of tests: reaction time (Dynavision™ D2), anticipation (Bassin Anticipation Timer), sustained attention (Go/No-Go and Eriksen Flanker Test) and memory tests. Moreover, the side effects and the perceived sensation index were recorded 24 h after each test.

Results Reaction time only improved following 6 mg/kg of caffeine intake (Physical reaction time: -0.04 s, 95% CI -0.08 to -0.01 s, $P=0.036$, $d=0.5$; Motor reaction time: -0.04 s, 95% CI -0.07 to -0.01 s, $P=0.008$, $d=0.6$) compared to the placebo condition. Anticipation, sustained attention, and memory were not affected after either caffeine dose intake (all $P>0.05$). In addition, the 6 mg/kg dose of caffeine augmented the occurrence of the side effects of increased activeness ($P=0.046$) and nervousness ($P=0.001$).

Conclusion Acute intake of 6 mg/kg caffeine is effective in improving reaction time despite increasing the occurrence of side effects in healthy physically active young adults.

Keywords: ergogenic aid, sports performance, reaction time, anticipation, attention, memory.

58 **DECLARATIONS**

59 **Conflict of interest** On behalf of all authors, the corresponding author states that there is no conflict of
60 interest

61 **Clinical Trial and Study Registration** This study has been registered in ClinicalTrials whose ID is:
62 NCT05995314.

63 **Ethical Approval** This study has been approved by the Research Ethics Committee of Francisco de Vitoria
64 University (nº 23/2023) and has therefore been performed in accordance with the ethical standards laid
65 down in the 1964 Declaration of Helsinki and its later amendments.

66 **Consent to participate** All participants provided written informed consent.

67 **Author contributions:** Introduction M.R-d, I.P-B and P.E-G; methodology and data collection A.B-S.,
68 P.E-G., M.R-d., I.P-B and P.A.; results A.B-S., M.R-d. and J.A-V.; discussion and conclusion M.R-d. and
69 P.A.; writing-preparation of the original draft M.R-d.; revision and editing J.A-V.; supervision J.A-V. All
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INTRODUCTION

Caffeine (1,3,7-trimethylxanthine) is one of the most widely consumed dietary ingredients in the world [1]. The main sources of caffeine are found in products such as brewed coffee, tea, cocoa products, and soft drinks [2]. In addition, caffeine can be artificially synthesised and is often included in a variety of medications, dietary supplements, and commercially available beverages [3], such as energy drinks, the consumption of which has increased enormously in recent years, especially in the sports context [2]. Caffeine is one of the most widely used supplements by athletes due to its effectiveness as an ergogenic aid, enhancing performance across a wide range of exercise modalities [4, 5] and in a variety of sport-specific tasks [6-8].

Most studies examining the effects of caffeine ingestion on exercise performance use a single dose of caffeine administered orally via capsules/tablets [9]. It is suggested that 3 mg/kg (body mass) is the optimal dose for ergogenic benefits in physical performance [10], also showing benefits with low doses (1-2 mg/kg) [11]. Caffeine intake of 3-6 mg/kg has also been shown to be effective in increasing physical [12, 13] and psychophysical performance [14, 15], but as the dose of caffeine is increased, the prevalence and magnitude of side effects increases [16]. High caffeine intake can lead to different adverse health effects [17, 18], such as tachycardia and nervousness [19], and gastrointestinal discomfort [20] which are the most common side effects at high doses. However, the magnitude and duration of these adverse effects could depend on the dose of caffeine ingested and interindividual biological variability [18, 21].

Caffeine is a powerful central nervous system stimulant and is mainly used to increase stamina and activity, prevent drowsiness, and restore alertness [2]. It has been shown that underlying mechanisms are dose-dependent. Low doses of caffeine (≤ 3 mg/kg) act mainly on the central nervous system [22, 23], increasing the activity of the prefrontal cortex and improving executive function [17, 24]. However, higher doses of caffeine (5-9 mg/kg) can cause peripheral effects [22], without affecting brain activation [24]. This was demonstrated in the study by Zhang et al. [25], where the effects of caffeine on cognition and brain activation were greater with low doses (3 mg/kg) of caffeine than with moderate (6 mg/kg) and high doses (9 mg/kg). Cognitive function training could provide significant benefits in some cognitive aspects such as memory, attention, cognitive processing speed, intelligence, fluency, problem-solving, and learning ability [26-28]. These cognitive aspects are associated with successful sport and exercise. Thus, cognitive function training could improve an athlete's sports performance [29], and its combination with caffeine intake could produce further enhancements.

Therefore, because cognitive performance is a cornerstone in sports associated with physical performance, and caffeine produces cognitive improvements, it is necessary to clarify the dose-response of caffeine on cognitive performance through sport-specific cognitive tests. Thus, the main purpose of this study was to determine the effects of different doses of caffeine (0, 3, 6 mg/kg) on cognitive aspects related to sport, in physically active and healthy people. We hypothesised that caffeine supplementation would increase cognitive performance assessed through the different tests used, with 3 mg/kg being the optimal dose.

METHODS

Participants

Twenty-nine healthy young adults (15 men and 14 women, age: 21.8 ± 1.8 years; height: 170.1 ± 8.9 cm, weight: 63.1 ± 10.8 kg) were recruited. Participants were physically active (i.e., >150 min/week moderate physical activity or >75 min/week vigorous physical activity [30]) and were not diagnosed with serious health problems. The exclusion criteria were suffering from colour blindness and/or regularly consuming caffeine (i.e., >3 cups of coffee/day [31]). In addition, all volunteers underwent screening (AHA/ACSM Health/Fitness Facility Preparticipation Screening Questionnaire [32]) to exclude individuals with any risk of cardiopulmonary disease and/or under any drug prescription. All subjects provided written, witnessed, and informed consent in accordance with a protocol approved by the local Ethics Committee and according to the Declaration of Helsinki.

Experimental design

All participants completed three trials in a double-blinded crossover randomised control trial. Then, their cognitive skills were evaluated under a) placebo (PLAC; 0 mg/kg); b) 3 mg/kg; or c) 6 mg/kg of body mass of caffeine. To achieve the blinded placebo situation, a researcher not associated with the study performed the blinding and prepared identical capsules containing placebo (cellulose) or the corresponding dose of caffeine (99% caffeine, Bulkpowders, United Kingdom). This researcher assigned an alphanumeric code to each trial to blind participants and investigators. The code was revealed after analysis of the variables. Treatment order was randomly allocated according to balanced permutations generated by a web-based computer programme (<http://www.randomization.com>) [33]. Trials were separated by at least 48-h, allowing full recovery and caffeine clearance [34, 35]. Participants were familiarised with the different cognitive tests through a study visit one week before trials. All study visits

were performed in the same thermoneutral environment ($20.4 \pm 0.5^\circ\text{C}$ and $31.2 \pm 1.3\%$ humidity). Participants were instructed to refrain from the consumption of any substance containing caffeine/alcohol (12h prior) [36] and to replicate their habitual physical activity levels and dietary practices before every trial [37]. Side effects and the perceived sensation index were recorded in the following 24-h after each experimental trial [2].

Experimental protocol

The first session served to familiarise participants with the instruments and tests that composed the cognitive test battery. In addition, participants were weighed (Seca 700, Germany) for subsequent individualisation of caffeine doses. In the next three experimental trials, their body weight was re-checked when the participants arrived at the laboratory. Then, they ingested their blind capsule with caffeine or placebo in front of the research team. This was done 60 minutes before the cognitive tests started since the caffeine plasma concentration peaked at 1 hour after ingestion [38]. Sixty minutes after ingestion, participants performed the battery of cognitive tests whose validity was evaluated in our recent pilot study [39] and where it is explained in greater detail. This battery (Fig. 1) was performed in the same order each day for each subject and consisted of measuring the following cognitive skills:

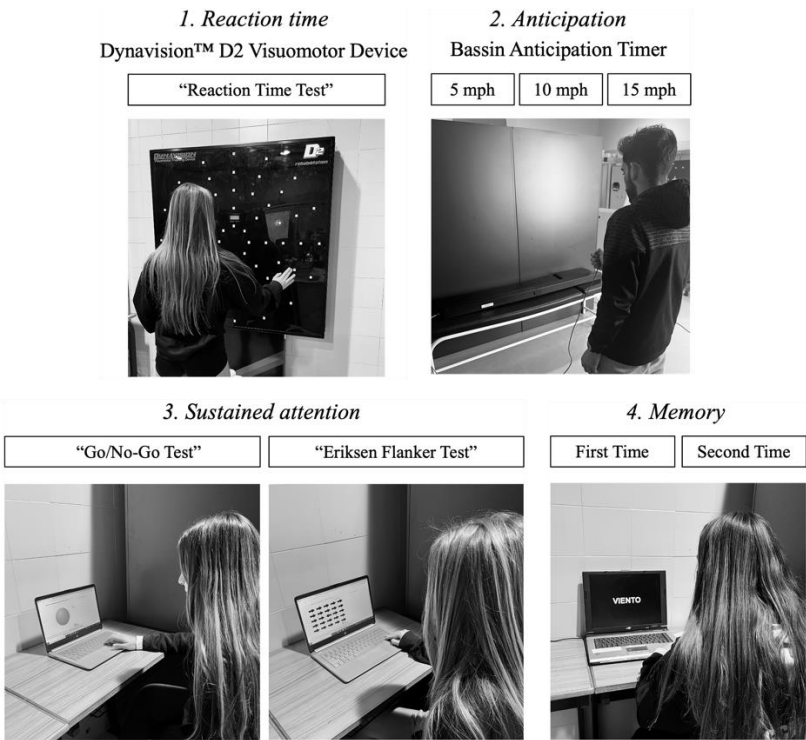


Fig. 1 Battery of cognitive tests used during all experiment trials

1. Reaction time

The Dynavision™ D2 Visuomotor Device was used to assess reaction time (RT; s) through the “Reaction Time Test” [40-43]. During the assessment, participants were located with the central screen at eye level and in a position that allowed them to comfortably press all the buttons on the device. Visual, motor, and physical reaction time (*VRT*, *MRT*, and *PRT*, respectively) were measured as the fastest RT (s) of three attempts with the dominant hand. *VRT* is the elapsed time from the appearance of the red light until the start button is released. *MRT* is the elapsed time from the release of the start button until the red light is pressed. *PRT* is the elapsed time from the appearance of the red light until the red light is pressed, i.e., *VRT* + *MRT*.

2. Anticipation

Visual acuity related to hand-eye coordination and anticipation was assessed using the Bassin Anticipation Timer [44]. Participants aimed to pulse the timer as close as possible to the goal light (light number 33). Three repetitions of each condition/speed (5, 10, and 15 miles per hour (*mph*) which is equivalent to 8, 16 and 24 kilometres per hour) were performed with a random signal delay. The best anticipation time (s) of each condition was used for the analysis.

3. Sustained attention

The ability of maintaining attention and inhibiting an appropriate response were measured by the “Go/No-Go Test” [45] and “Eriksen Flanker Test” [46]. Both tests were performed through a web-based computer programme (<http://cognitivefun.net/>) used by previous studies to measure cognitive function [46, 47]. Participants during the “Go/No-Go Test” aimed to react with their dominant hand as quickly as possible when the “Go” stimulus appeared, holding their response when the “No-Go” stimulus was presented. The *Fastest*, the *Slowest*, and the *Average* response time (s), and successful attempts (*Percentage*; %) were used for the following statistical analysis. During the “Eriksen Flanker Test”, participants also aimed to react with their dominant hand to the direction of the target arrow that appeared in the centre of the screen. This target arrow appeared surrounded by other arrows, oriented congruently (same direction) or incongruently (opposite direction). The congruent (*CRT*) and incongruent (*IRT*) reaction time (s) were measured.

4. Memory

Participants performed a similar test to that developed by Nielson et al. [48] to study the modulation of memory storage processes. Three 20-item word lists were created by selecting concrete and imageable nouns (ratings 6.49; scale = 1 (low) to 7 (high)) from the normative list of Paivio et al. [49]. Each word on the list was presented for 5 s. This was followed by a memory consolidation interval of 100

s, after which there was a 120 s period in which the participants wrote as many words as they could remember (*First Time*). After 15 minutes, the participants performed a recall task (*Second Time*) in which they had to rewrite all the words they could remember at the time. The number of correct words recalled was analysed regardless of their order of presentation and was considered correct if there were small typographical errors or singular-plural substitutions.

Statistical analysis

All statistical procedures were run on SPSS version 28 (IBM Corp., Armonk, N.Y., USA). The Shapiro–Wilk test revealed that data were normally distributed. A repeated-measures ANOVA was used to identify differences between experimental trials followed by the Bonferroni post hoc test when a significant interaction was detected. Effect size was obtained using Cohen’s *d* for pairwise comparison, with effects considered large if ≥ 0.8 , moderate ≥ 0.5 , and small if < 0.5 [50]. Cochran’s *Q* test was used to analyse differences on the self-reported side effects between experimental trials. Sample size calculation was based on Zhang et al. [25] who compared three doses of caffeine (3, 6, 9 mg/kg) on RT in healthy individuals.

The following formula was used to calculate the sample size: $n = \frac{2 \cdot (Z_{\alpha/2} + Z_{1-\beta})^2 \cdot \sigma^2}{d^2}$. Where *n* is the minimum number of subjects, $Z_{\alpha/2}$ is the standard deviation for a type 1 error (for a significance level of 5%, $Z_{\alpha/2} = 1.96$), $Z_{1-\beta}$ is the standard deviation for the power of the study (for a statistical power of 80%, $Z_{1-\beta} = 1.28$), σ is the pooled standard deviation and *d* is the mean difference. It has been calculated that the minimum number of participants needed to reach a power of 0.8 and a bilateral α -level of 0.05 was 16 participants. Data in text and tables are reported as mean $\pm$ SD, and as mean $\pm$ SEM in Fig. 2. Ninety-five percent confidence intervals (CIs) were also calculated. The significance level for all statistical tests was $P < 0.05$.

RESULTS

Reaction Time

The RT assessed by the Dynavision™ D2 Visuomotor Device are depicted in Fig. 2. Although VRT was not affected by caffeine intake ($P = 0.384$), a caffeine effect was found on *MRT* and *PRT* ($P = 0.004$, and $P = 0.041$, respectively). Moreover, *PRT* and *MRT* only improved after the intake of 6 mg/kg of caffeine (*PRT*: mean difference (MD) -0.04 s, 95% confidence interval (CI) -0.08 to -0.01 s, $P = 0.036$, $d = 0.5$; *MRT*: MD -0.04 s, 95% CI -0.07 to -0.01 s, $P = 0.008$, $d = 0.6$) compared to the placebo condition. Despite the effect sizes observed, *PRT* and *MRT* did not improve following 3 mg/kg of caffeine (*PRT*: MD

-0.03 s, 95% CI -0.08 to 0.02 s, $P = 0.314$, $d = 0.3$; *MRT*: MD -0.02 s, 95% CI -0.06 to 0.01 s, $P = 0.135$, $d = 0.4$) compared to the placebo condition.

Anticipation

Visual acuity assessed using the Bassin Anticipation Timer and related to hand-eye coordination and anticipation was not affected after the intake of caffeine (Table 1) during 5 *mph* ($P = 0.756$), 10 *mph* ($P = 0.532$) or 15 *mph* ($P = 0.466$) conditions/speeds.

Sustained attention

Sustained attention measured through the “Go/No-Go Test” and “Eriksen Flanker Test” is also depicted in Table 1. In the “Go/No-Go Test”, significant effects were not observed following caffeine intake on *Fastest* ($P = 0.283$), *Slowest* ($P = 0.116$); and *Average* ($P = 0.519$) response time. Moreover, the successful attempts (*Percentage*: $P = 0.831$) were not affected by caffeine doses. Nevertheless, 6 mg/kg presented a small effect on improving the *Fastest* (MD -0.01 s, 95% CI -0.03 to 0.01 s; $P = 0.412$, $d = 0.3$) and the *Slowest* (MD -0.033 s, 95% CI -0.093 to 0.025 s; $P = 0.471$, $d = 0.3$) response times compared to 3 mg/kg. In the “Eriksen Flanker Test”, *CRT* was affected by caffeine ingestion ($P = 0.035$), but 3 or 6 mg/kg doses of caffeine did not significantly lower *CRT* (3 mg/kg: MD -0.02 s, 95% CI -0.03 to 0.01 s, $P = 0.064$, $d = 0.5$; 6 mg/kg: MD -0.05 s, 95% CI -0.03 to 0.02 s, $P = >0.999$, $d = 0.1$) compared to the placebo condition (0 mg/kg). A significant caffeine effect was not observed on the *IRT* ($P = 0.435$).

Memory

The results of both attempts at the memory test in each trial are described in Table 1. Despite the differences observed between trials, the number of correct words recalled was not improved following ingestion of 3 or 6 mg/kg of caffeine during both attempts (*First Time*, $P = 0.730$; *Second Time*, $P = 0.228$). During the *First Time* neither dose of caffeine showed any effect (3 mg/kg: MD 0.03 words, 95% CI -1.6 to 1.7 words, $P = >0.999$, $d = -0.1$; 6 mg/kg: MD 0.38 words, 95% CI -1.3 to 2.1 words, $P = >0.999$, $d = -0.1$). Nevertheless, the dose of 6 mg/kg presented a small effect on improving memory during the *Second Time* compared to the placebo (MD 1.28 words, 95% CI -0.6 to 3.2 words; $P = 0.297$, $d = 0.3$) and 3 mg/kg of caffeine (MD 0.72 words, 95% CI -0.6 to 2.1 s; $P = 0.536$, $d = 0.3$).

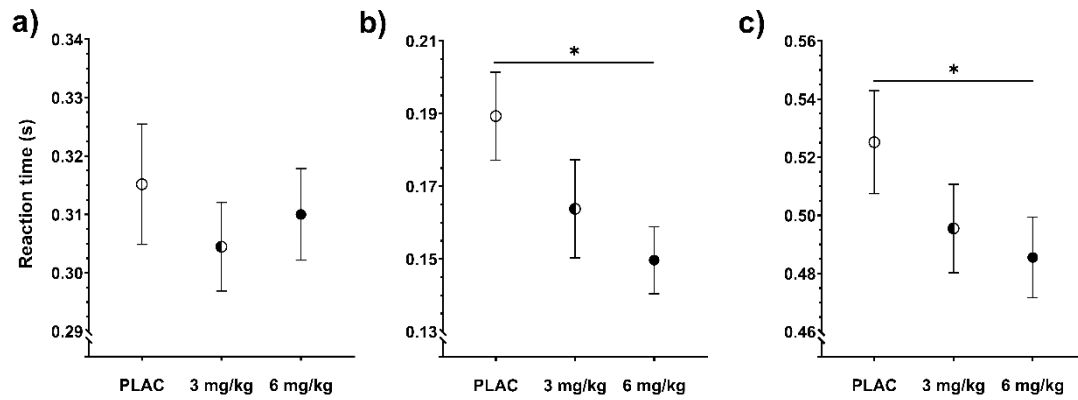


Fig. 2 Dose–response effects of caffeine ingestion on reaction time in its variables: a) Visual Reaction Time, b) Motor Reaction Time and c) Physical Reaction Time. *Significant difference ($P \leq 0.05$). PLAC: Placebo

246 **Table 1.** Cognitive response under placebo, 3 mg/kg, or 6 mg/kg of body mass of caffeine.

TEST	VARIABLES	PLAC	3mg/kg	6mg/kg	ANOVA	PLAC vs. 3mg/kg (Effect size)	PLAC vs. 6mg/kg (Effect size)	3mg/kg vs. 6mg/kg (Effect size)	
Anticipation	5 mph (s)	0.030±0.028	0.032±0.023	0.037±0.040	0.756	>0.999 (-0.1)	>0.999 (-0.1)	>0.999 (-0.1)	
	10 mph (s)	0.041±0.044	0.051±0.038	0.047±0.042	0.532	0.877 (-0.2)	>0.999 (-0.2)	>0.999 (0.1)	
	15 mph (s)	0.083±0.068	0.070±0.055	0.077±0.058	0.466	0.637 (0.2)	>0.999 (0.1)	>0.999 (-0.1)	
Sustained attention	Fastest (s)	0.328±0.044	0.333±0.043	0.322±0.036	0.283	>0.999 (-0.2)	>0.999 (0.1)	0.412 (0.3)	
	Slowest (s)	0.456±0.077	0.493±0.089	0.459±0.097	0.116	0.109 (-0.4)	>0.999 (-0.1)	0.471 (0.3)	
	Average (s)	0.384±0.043	0.392±0.061	0.380±0.049	0.519	>0.999 (-0.2)	>0.999 (0.1)	0.799 (0.2)	
	Percentage (%)	95.16±8.70	96.21±5.77	96.01±6.98	0.831	>0.999 (-0.1)	>0.999 (-0.1)	>0.999 (0.1)	
	“Eriksen Flanker Test”	CRT (s)	0.440±0.044	0.426±0.037	0.435±0.041	0.035	0.064 (0.5)	>0.999 (0.1)	0.399 (-0.2)
		IRT (s)	0.484±0.058	0.471±0.056	0.472±0.033	0.435	0.594 (0.2)	0.867 (0.2)	>0.999 (-0.1)
	Memory test	First Time (words)	11.55±2.65	11.58±3.33	11.93±3.30	0.730	>0.999 (-0.1)	>0.999 (-0.1)	>0.999 (-0.1)
		Second Time (words)	10.27±3.27	10.82±3.79	11.55±3.58	0.228	>0.999 (-0.2)	0.297 (-0.3)	0.536 (-0.3)

CRT: Congruent Reaction Time; IRT: Incongruent Reaction Time; mph: miles per hour; PLAC: Placebo.

Side Effects and Self-reported Perception

The side effects reported by the participants are shown in Table 2. The dose of 6 mg/kg of caffeine increased the appearance of the side effects of increased activeness ($P=0.046$) and nervousness ($P=0.001$) following intake, meanwhile, the dose of 3 mg/kg did not increase these side effects ($P=0.166$ and $P=0.058$, respectively). No other side effects were reported during trials (all $P>0.05$). In addition, the self-reported perception was not altered by the intake of 3 or 6 mg/kg of caffeine (all $P>0.05$; Table 3).

Table 2. Side effects reported during the different trials.

Side Effects	Caffeine Doses		
	Placebo	3 mg/kg	6 mg/kg
Headache	2 (6.90%)	2 (6.90%)	1 (3.45%)
Abdominal discomfort	1 (3.45%)	1 (3.45%)	0
Muscle soreness	0	0	0
Increased activeness	12 (41.38%)	17 (58.62%)	20 (68.97%) *
Tachycardia and/or palpitations	1 (3.45%)	1 (3.45%)	4 (13.79%)
Insomnia	3 (10.34%)	3 (10.34%)	7 (24.14%)
Increased urine production	4 (13.79%)	6 (20.69%)	8 (27.59%)
Increased anxiety	2 (6.90%)	3 (10.34%)	8 (27.59%)
Nervousness	4 (13.79%)	10 (34.48%)	15 (51.72%) *
Gastrointestinal problems	2 (6.90%)	3 (10.34%)	3 (10.34%)
Irritability	1 (3.45%)	0	0

Data are presented as the number of individuals (n) and the percentage of prevalence (%).

*Significant different ($P<0.05$) from placebo condition

Table 3. Self-reported perception during trials.

Self-reported Perception	Caffeine Doses			ANOVA
	Placebo	3 mg/kg	6 mg/kg	
Reaction time	7.55±1.27	7.14±1.52	7.48±1.59	0.505
Anticipation	7.14±1.35	6.93±1.51	7.00±1.62	0.823
Sustained attention	6.90±1.51	7.17±1.67	7.21±1.47	0.693
Memory	6.45±1.76	6.72±1.84	6.93±1.86	0.541

Data are presented as the mean ± SD of the evaluation from 0 to 10 (0 = “none” or 10 = “the maximum”) for each cognitive variable analysed.

DISCUSSION

Caffeine is the most widely used ergogenic supplement in sports [51]. Although its physiological effects have been described, its effects on cognitive function are still unclear. Our study found that only 6 mg/kg of caffeine improved RT (i.e., lower motor and physical reaction times) measured by the Dynavision™ D2 Visuomotor Device in healthy young individuals. Nevertheless, we found no significant differences in other cognitive skills (anticipation, attention, and/or memory). The improvements in RT are explained by an increase in whole-body muscular activity, which may be related to improved sports performance. On the other hand, we found that 6 mg/kg of caffeine augmented the occurrence of side effects, namely increased activeness, and nervousness. Thus, despite increasing side effects, moderate doses of caffeine intake do not worsen cognitive function, and even beneficial results are obtained that could improve sports performance.

RT is one of the most important qualities for a sports practitioner [52]. It is suggested that caffeine intake could have an ergogenic effect reducing RT [4]. Balko et al. [53], comparing different doses of caffeine, found that the consumption of 3 mg/kg of caffeine positively affects RT. Moreover, RT could improve sport-specific tasks as observed in studies with athletes [54-56]. Souissi et al. [55] found that 5 mg/kg dose of caffeine improves RT during a task of responding to a visual stimulus by pressing a key in elite judo athletes. The study by Santos et al. [56] also found an improvement in RT during a sport-specific task in taekwondists. In our study, we showed that 6 mg/kg of caffeine reduced RT during a cognitive test, but 3 mg/kg did not improve it. This positive effect of caffeine on RT may be related to increased arousal, as caffeine activates pathways that have traditionally been associated with motor responses in the brain [56]. We also believe that this improvement in RT may be more related to the physical than the cognitive performance since our RT test involved rapid muscle activity to score well. Thus, caffeine supplementation improves RT during sport-related tasks, enhancing performance.

Anticipation time is defined as the estimation of arrival of a stimulus and the required time for a response to intercept it [57]. Anticipation contributes to success in numerous tasks in daily life and sport [58], such as hitting a moving object, with lack of accuracy in anticipation being the main reason for technical or tactical errors during competition [59]. The Bassin Anticipation Timer is the most accurate, reliable and validated way to measure anticipation [60]. Duncan et al. [61] and Tallis et al. [62] found that caffeine improved (i.e., vs. the placebo condition) anticipation in older regular coffee consumers at 5mph and 8mph speeds, respectively. Nevertheless, it should be noted that the effects of caffeine on anticipatory

processing are quite specific [63], and the assessment of anticipation will have greater construct validity, when the interceptive task is sport-specific [64]. Clarke and Duncan [59] examined a sport population (badminton players) and found that caffeine improved anticipation at 3 and 5 mph speeds, improving badminton performance. We did not find any improvement of caffeine on anticipation (i.e., 5, 10, and 15 mph) in physically active healthy individuals. Since faster target movement speeds were associated with worse dynamic visual acuity (i.e., anticipation) in both caffeine and placebo conditions [65], the higher speeds used can explain our lack of significant findings.

There is controversy on the effects of caffeine on attention. Some studies suggested that caffeine improves sustained attention [66], and performance on simple and complex attention tasks [67] which could be attributed to its antagonistic effect on the adenosine receptor [66]. However, other studies did not support a significant caffeine effect on attention [68, 69]. Pasman et al. [70] showed small improvements after caffeine intake (85 mg) in the Go/No-Go test results in healthy adults. On the contrary, neither CRT nor IRT during the Eriksen Flanker test were affected by caffeine supplementation (3 mg/kg) in healthy middle-aged [71] and older adults [72]. High doses of caffeine ingestion are sometimes associated with detrimental effects on performance, due to distraction [73] or their side effects. In our study, even though participants manifested some side effects, attention was not negatively affected and could even be improved with moderate doses of caffeine (6 mg/kg). Therefore, further studies are needed to clarify the effects of caffeine on attention.

As a nootropic drug, caffeine may be related to the enhancement of cognitive resources in memory processes [74], but there is no reliable evidence that an experimentally administered dose of caffeine can have an effect on short-term memory performance, at least in healthy individuals [74]. In our research, although we found a tendency towards better results in the memory test the higher the caffeine dose, we could not clearly conclude the effects of caffeine on memory, as the results obtained were not statistically significant. In agreement, several studies did not find significant changes in short-term memory after caffeine supplementation compared to a placebo using a similar test to ours [75, 76]. Therefore, caffeine may not have a reliable, unidirectional effect on performance in immediate recall tasks (i.e., short-term memory), but it could have some positive effects on measures of long-term memory [74].

The side effects of high ingested doses of caffeine are well known, being the most frequent tachycardia and palpitations, nervousness, increased urine production, gastrointestinal problems, headache, insomnia, and muscle soreness [10]. In our study we evaluated these side effects and added the following effects

proposed by Salinero et al. [2] in his study: abdominal discomfort, irritability, and increased activeness and anxiety. Our results indicated that caffeine intake at the highest dose (6 mg/kg) increased activeness and nervousness, which would agree with the findings of Pallares et al. [77] and Salinero et al. [2]. This outcome is interesting to comment on because both are side effects that are not associated with detrimental effect on sports performance [78]. Regarding reported self-perception, our trend showing better perception of sustained attention and memory with the higher caffeine dose was not significant, maybe because it can differ greatly between individuals [21]. Therefore, caffeine, although it may cause some side effects, does not have a detrimental effect on sports performance, and may even improve it.

Our study is not free of limitations. The cognitive variables studied could be influenced by the measurement systems and procedures. Moreover, we only used low (3 mg/kg) and moderate (6 mg/kg) caffeine doses compared to other studies. A higher dose of caffeine (i.e., 9 mg/kg) may be able to produce more significant results versus the placebo condition. Another aspect to consider is individual differences in genetics and caffeine sensitivity. Although a high daily coffee intake (i.e., >3 cups of coffee/day) was an exclusion criterion, it is possible that greater effects can be presented in participants with less familiarity with caffeine. On the other hand, they were all physically active young adults with a presumed good cognitive function which may have led to smaller effects and therefore insignificant improvements in the cognitive variables measured than if we had studied another type of population (i.e., older adults, non-athletes, people with health problems, etc.). Regarding genetics, we did not take them into account and it would have been nice to assess them since there is a hypothesis that genes may differentially affect adenosine metabolism [71], which has a major impact on the CNS [61].

PRACTICAL APPLICATION

RT is a determining aspect in sports performance or even in achieving victory. Our study suggests that ingestion of a moderate dose of caffeine (6 mg/kg body mass) is a potent ergogenic aid for improving RT in healthy physically active individuals. Therefore, caffeine doses of 6 mg/kg body weight could be considered by coaches and athletes who want to lower RT and improve in their sports discipline. In addition, although a dose of 6 mg/kg may increase the occurrence of side effects, such as nervousness or activeness, performance would not be affected following ingestion. Therefore, the dose of 6mg/kg may be sufficient to improve RT and not produce harmful side effects.

CONCLUSIONS

In summary, the acute intake of 6 mg/kg of caffeine was effective in improving RT in the test used on the Dynavision™ D2 visuomotor device. In addition, the 6 mg/kg dose of caffeine augmented the occurrence of side effects, mainly increased activeness and nervousness. Although no significant differences were found in the other cognitive skills studied (anticipation, attention, and/or memory) between the doses of caffeine studied and the placebo condition, we propose to continue investigating with a higher dose of caffeine and with a different protocol for measuring cognitive abilities, since there were trends of improvement in cognitive performance when the dose of caffeine was higher, but without showing significant results.

DECLARATIONS

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical Approval This study has been approved by the Research Ethics Committee of Francisco de Vitoria University (n° 23/2023) and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent to participate All participants provided written informed consent.

HIGHLIGHTS

- Although caffeine is considered ergogenic at doses of 3 to 6 mg/kg body mass, we have only found significant differences with the 6 mg/kg dose.
- The acute intake of 6 mg/kg body mass of caffeine is effective in improving reaction time.
- The 6 mg/kg dose of caffeine augmented the occurrence of side effects, mainly increased activeness and nervousness.
- Caffeine can be a potent ergogenic aid to improve sports performance (i.e., motor and physical reaction time), however, at the cognitive level no improvements have been found with the doses used (3 and 6 mg/kg).
- Further research with higher doses of caffeine (i.e., 9 mg/kg) and with different protocols for measuring cognitive abilities is needed to test whether the trends shown in our study could be converted into real improvements in cognitive performance.

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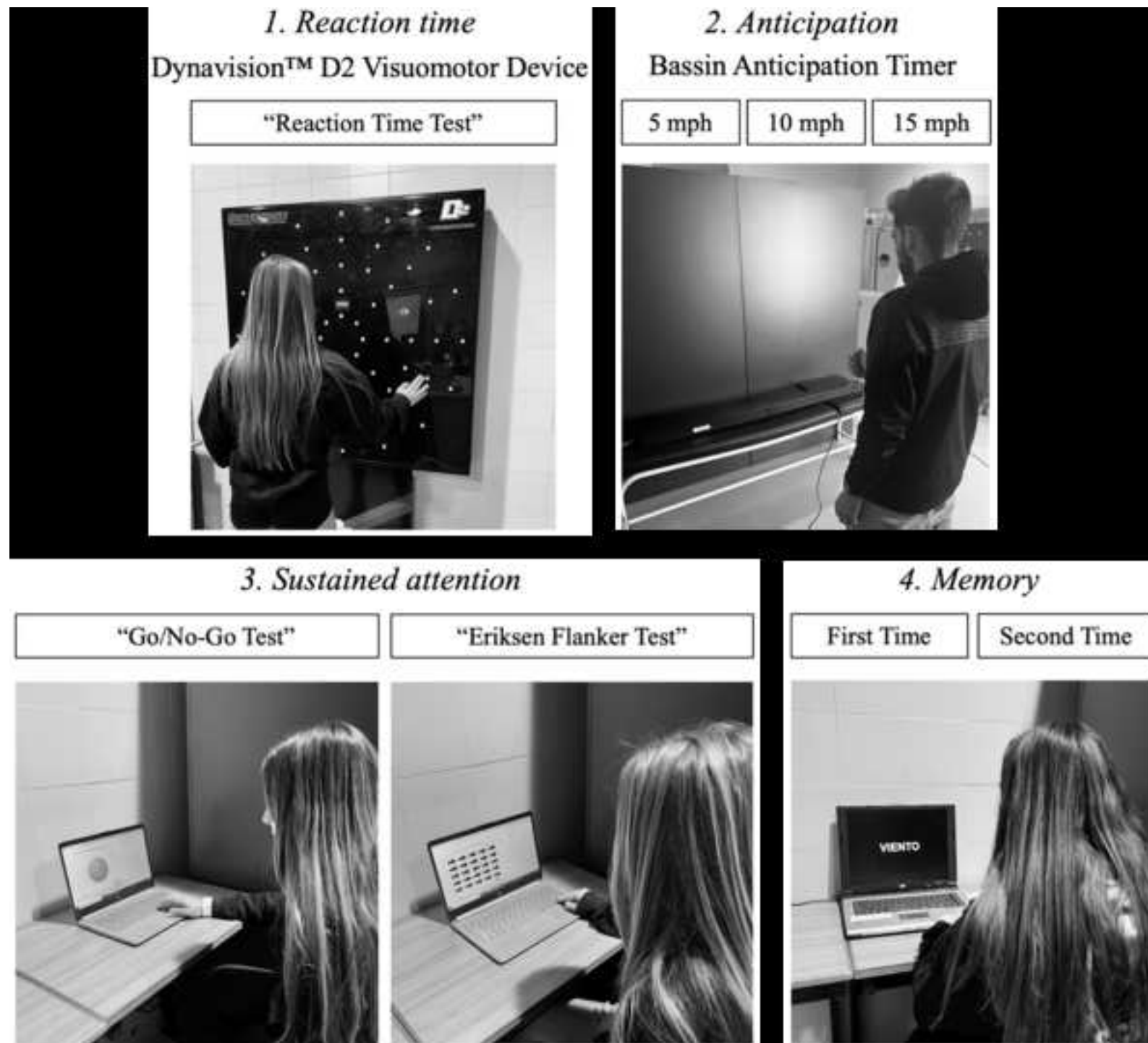
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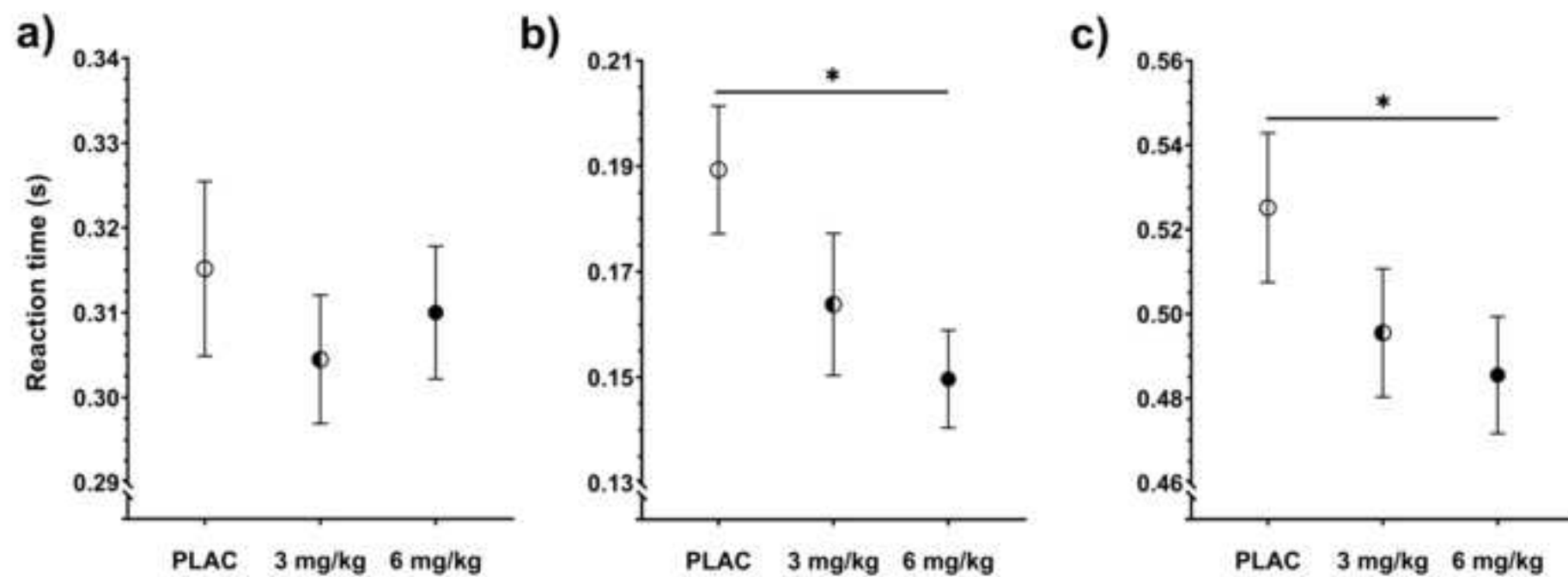


Table 1

Table 1. Cognitive response under placebo, 3 mg/kg, or 6 mg/kg of body mass of caffeine.

TEST	VARIABLES	PLAC	3mg/kg	6mg/kg	ANOVA	PLAC vs. 3mg/kg (Effect size)	PLAC vs. 6mg/kg (Effect size)	3mg/kg vs. 6mg/kg (Effect size)	
Anticipation	5 mph (s)	0.030±0.028	0.032±0.023	0.037±0.040	0.756	>0.999 (-0.1)	>0.999 (-0.1)	>0.999 (-0.1)	
	10 mph (s)	0.041±0.044	0.051±0.038	0.047±0.042	0.532	0.877 (-0.2)	>0.999 (-0.2)	>0.999 (0.1)	
	15 mph (s)	0.083±0.068	0.070±0.055	0.077±0.058	0.466	0.637 (0.2)	>0.999 (0.1)	>0.999 (-0.1)	
Sustained attention	Fastest (s)	0.328±0.044	0.333±0.043	0.322±0.036	0.283	>0.999 (-0.2)	>0.999 (0.1)	0.412 (0.3)	
	Slowest (s)	0.456±0.077	0.493±0.089	0.459±0.097	0.116	0.109 (-0.4)	>0.999 (-0.1)	0.471 (0.3)	
	Average (s)	0.384±0.043	0.392±0.061	0.380±0.049	0.519	>0.999 (-0.2)	>0.999 (0.1)	0.799 (0.2)	
	Percentage (%)	95.16±8.70	96.21±5.77	96.01±6.98	0.831	>0.999 (-0.1)	>0.999 (-0.1)	>0.999 (0.1)	
	“Eriksen Flanker Test”	CRT (s)	0.440±0.044	0.426±0.037	0.435±0.041	0.035	0.064 (0.5)	>0.999 (0.1)	0.399 (-0.2)
		IRT (s)	0.484±0.058	0.471±0.056	0.472±0.033	0.435	0.594 (0.2)	0.867 (0.2)	>0.999 (-0.1)
	Memory test	First Time (words)	11.55±2.65	11.58±3.33	11.93±3.30	0.730	>0.999 (-0.1)	>0.999 (-0.1)	>0.999 (-0.1)
		Second Time (words)	10.27±3.27	10.82±3.79	11.55±3.58	0.228	>0.999 (-0.2)	0.297 (-0.3)	0.536 (-0.3)

CRT: Congruent Reaction Time; IRT: Incongruent Reaction Time; mph: miles per hour; PLAC: Placebo.

Table 2. Side effects reported during the different trials.

Side Effects	Caffeine Doses		
	Placebo	3 mg/kg	6 mg/kg
Headache	2 (6.90%)	2 (6.90%)	1 (3.45%)
Abdominal discomfort	1 (3.45%)	1 (3.45%)	0
Muscle soreness	0	0	0
Increased activeness	12 (41.38%)	17 (58.62%)	20 (68.97%) *
Tachycardia and/or palpitations	1 (3.45%)	1 (3.45%)	4 (13.79%)
Insomnia	3 (10.34%)	3 (10.34%)	7 (24.14%)
Increased urine production	4 (13.79%)	6 (20.69%)	8 (27.59%)
Increased anxiety	2 (6.90%)	3 (10.34%)	8 (27.59%)
Nervousness	4 (13.79%)	10 (34.48%)	15 (51.72%) *
Gastrointestinal problems	2 (6.90%)	3 (10.34%)	3 (10.34%)
Irritability	1 (3.45%)	0	0

Data are presented as the number of individuals (n) and the percentage of prevalence (%).

*Significant different (P<0.05) from placebo condition

Table 3. *Self-reported perception during trials.*

Self-reported Perception	Caffeine Doses			ANOVA
	Placebo	3 mg/kg	6 mg/kg	
Reaction time	7.55±1.27	7.14±1.52	7.48±1.59	0.505
Anticipation	7.14±1.35	6.93±1.51	7.00±1.62	0.823
Sustained attention	6.90±1.51	7.17±1.67	7.21±1.47	0.693
Memory	6.45±1.76	6.72±1.84	6.93±1.86	0.541

Data are presented as the mean ± SD of the evaluation from 0 to 10 (0 = “none” or 10 = “the maximum”) for each cognitive variable analysed.