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3 **Article Title: Dispelling the myth that habitual caffeine consumption influences the performance**
4 **response to acute caffeine supplementation**

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26 **ABSTRACT**

27 **Objective:** To investigate the influence of habitual caffeine intake on aerobic exercise performance responses
28 to acute caffeine supplementation. **Methods:** A double-blind, crossover, counterbalanced study was performed.
29 Forty male endurance-trained cyclists were allocated into tertiles according to their daily caffeine intake: low
30 ($58 \pm 29 \text{ mg}\cdot\text{d}^{-1}$), moderate ($143 \pm 25 \text{ mg}\cdot\text{d}^{-1}$), and high consumers ($351 \pm 139 \text{ mg}\cdot\text{d}^{-1}$). Participants completed
31 three trials in which they performed simulated cycling time-trials in the fastest time possible following
32 ingestion of: caffeine (CAF: $6 \text{ mg}\cdot\text{kg}^{-1}$ BM), placebo (PLA), and no supplement (CON). **Results:** Mixed-model
33 analysis revealed time-trial performance was significantly improved in CAF compared to PLA and CON
34 (29.92 ± 2.18 min vs 30.81 ± 2.67 and 31.14 ± 2.71 min; $P = <0.0002$). ANCOVA revealed no influence of
35 habitual caffeine intake as a covariate on exercise performance ($P=0.47$). Time-trial performance was not
36 significantly different between tertiles ($P=0.75$). No correlation was observed between habitual caffeine intake
37 and absolute changes (CAF – CON) in time-trial performance with caffeine ($P=0.524$). Individual analysis
38 showed that eight, seven and five individuals improved above the variation of the test in CAF in the low,
39 moderate and high tertiles, respectively. A Fisher's Exact Test did not show any significant differences in the
40 number of individuals who improved in CAF between the tertiles ($P>0.05$). Blood lactate and ratings of
41 perceived exertion were not different between trials and tertiles ($P>0.05$). **Conclusion:** Performance effects of
42 acute caffeine supplementation during a ~30 min cycling TT performance were not influenced by the level of
43 habitual caffeine consumption.

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45 **Keywords:** supplement; daily consumption; endurance; time-trial.

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49 New & Noteworthy

50 ✓ There has been a long-standing paradigm that habitual caffeine intake may influence the ergogenicity of
51 caffeine supplementation.

52 ✓ Low, moderate and high caffeine consumers showed similar absolute and relative improvements in cycling
53 time-trial performance following acute supplementation of 6 mg·kg⁻¹ BM caffeine.

54 ✓ Performance effects of acute caffeine were not influenced by the level of habitual caffeine consumption,
55 suggesting that high habitual caffeine intake does not negate the benefits of acute caffeine supplementation.

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75 **INTRODUCTION**

77 The most consumed psychoactive substance worldwide, caffeine, is a xanthine-derived alkaloid (1,3,7-
78 trimethylxanthine) naturally found in common dietary products, such as coffee, green-tea extracts, chocolate,
79 and soda.(8) Ingestion of 5-6 mg·kg⁻¹BM caffeine results in peak plasma caffeine concentrations of 30-49
80 μmol·L⁻¹ within 40 to 60 minutes,(17) and its half-life may vary from 3 to 6 hours.(5) Caffeine use in sports is
81 highly prevalent and supported by meta-analytic data, which has been shown that a median dose of 6 mg·kg⁻¹
82 ¹BM 60 min before exercise h improves performance (+1.9%; 95% CI: 0.01% to 3.8%) during both endurance
83 and high-intensity exercise protocols.(11)

84 The exact mechanisms by which caffeine exerts its ergogenic effects are still under debate, with
85 suggested mechanisms including fatty acid mobilization and oxidation, endogenous glycogen content sparing,
86 calcium ion release from the sarcoplasmic reticulum and potassium ion attenuation in the interstitium (for
87 reviews see. (16, 24) However, its ergogenic effects are most likely due to its ability to act as an adenosine A₁
88 and A_{2A} receptor antagonist, ultimately resulting in increased dopamine and noradrenaline release, thereby
89 promoting feelings of wakefulness and alertness, and decreasing the rate of perceived exertion and pain during
90 exercise.(27) Regular consumption of caffeine has been associated with an upregulation of the number of
91 adenosine receptors in vascular and neural tissues of the brain.(12, 13) Based on these observations, it could be
92 speculated that habitual and non-habitual caffeine consumers would respond differently to caffeine
93 supplementation during exercise. Indeed, Beaumont, et al.(4) recently demonstrated that 4 weeks of caffeine
94 ingestion (1.5 – 3.0 mg·kg⁻¹·day⁻¹) resulted in an increased tolerance to acute caffeine supplementation in
95 previously low habitual caffeine consumers, with the ergogenic effect of acute caffeine supplementation no
96 longer apparent. Based upon these data, it appears reasonable to assume that individuals with different levels of
97 long-term habitual caffeine consumption (i.e., low, moderate or high) may also show differences in the
98 ergogenic response to acute caffeine supplementation.

99 Studies directly addressing the relationship between habitual intake of caffeine and the effect of caffeine
00 supplementation on exercise performance are inconsistent. Dodd, et al.(10) showed no differences in the effects
01 of caffeine on exercise performance between caffeine naïve individuals and habitual consumers. Conversely,
02 Bell & McLellan(5) demonstrated that non-habitual caffeine consumers cycled longer than habitual consumers
03 during a cycling-to-exhaustion protocol at 80% VO_{2max} following acute caffeine ingestion. These studies are

04 inconclusive due to several factors including their binary stratification of subjects as habitual ($> 300 \text{ mg day}^{-1}$)
05 or non-habitual consumers ($< 50 \text{ mg day}^{-1}$), disregarding intermediary intakes. Moreover, these studies enrolled
06 a relatively low total number of subjects ($n < 21$), hampering definitive conclusions. Finally, to assess
07 performance, time-to-exhaustion tests were employed, which have been subjected to criticisms due to poor
08 external validity and large variability.(9, 20) Notwithstanding the lack of solid evidence, there is a belief that
09 individuals who have a low caffeine consumption may have greater performance improvements following acute
10 caffeine supplementation than those who have a high caffeine consumption, which has led to practical
11 recommendations such as removing/reducing caffeine from the diet before supplementing with caffeine for
12 sports competition.(31)

13 In light of these contrasting data, this study aimed to investigate whether the long-standing notion holds
14 true that habitual intake of caffeine (i.e., low, moderate, and high) influences the effects of acute caffeine
15 supplementation on exercise performance using a large sample of volunteers and a reliable endurance exercise
16 protocol. We hypothesized that habitual caffeine intake would influence the ergogenic effects of caffeine
17 supplementation, with greater aerobic exercise performance gains in individuals with lower regular
18 consumption.

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22 MATERIALS AND METHODS

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24 *Participants*

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26 Forty-two recreationally-trained male cyclists, out of 125 screened, participated in this study, although
27 only forty were included in the analysis since one individual did not complete the habitual intake form and
28 another did not complete all exercise trials for reasons unrelated to the study. All of them were competitive
29 cyclists at either amateur or professional level, trained at least 4 times/week, and cycled over 150 km/week. The
30 exclusion criteria included the use of any dietary supplement (except carbohydrate and proteins) for at least 6
31 months prior to the study, and any prior use of anabolic steroids. Participants were fully informed of the nature
32 and possible risks of the experimental procedures before their written consent was obtained. The study was
33 approved by the University of São Paulo's Ethics Review Committee as part of a larger thematic project, the
34 remaining data of which are reported elsewhere (Saunders et al., in press).(30)

35

36 *Experimental design*

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38 This was a double-blind, counterbalanced, crossover study. Participants underwent three experimental
39 trials: caffeine supplementation (CAF), placebo supplementation (PLA) and no supplement (CON). The
40 experimental sessions were performed on different days at least 7 days apart. Participants were randomly
41 assigned to the experimental trials using a Latin Square model.(25)

42 Participants visited our laboratory on six separate occasions. On the first day, height and body mass
43 were measured, and individuals responded to a validated Food Frequency Questionnaire (FFQ) to assess
44 habitual caffeine intake. Thereafter, a maximal incremental test was performed to determine VO_{2max} . On days 2
45 and 3, familiarizations to the main exercise test were performed. On days 4, 5, and 6, participants engaged in
46 the main experimental trials, which consisted of a simulated cycling time-trial (TT) performed on an
47 electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). All exercise tests were
48 individually standardized to be carried out at the same time of day following a 6-h fasting period and 60-min
49 after supplement ingestion.

50 To tightly control this study, participants were instructed to abstain from training, alcohol and caffeine-
51 containing substances within the 24-h period prior to the experimental sessions. Participants were also asked to
52 record a 24-h food recall before each test, from which carbohydrate, protein, fat and caloric intake were
53 calculated. To assist the participants in refraining from dietary caffeine, they were provided with a
54 comprehensive list of the main products containing caffeine. Additionally, the participants were given a book
55 with instructions and illustrative examples on how to fill out the dietary recall. The food recalls were analyzed
56 before each experimental session by a member of our team to ensure that participants did not consume any food
57 or beverage containing caffeine.

58 To test the influence of habitual caffeine intake on the exercise responses to acute caffeine
59 supplementation, participants were allocated into tertiles according to their dietary intake of caffeine using
60 statistical software (STATISTICA v.10; StatSoft, Tulsa, USA). This resulted in the following sub-groups: low
61 consumers ($58 \pm 29 \text{ mg}\cdot\text{day}^{-1}$; range = 2 to 101 $\text{mg}\cdot\text{day}^{-1}$; 95% CI: 44.2 – 71.7; $n = 14$), moderate consumers
62 ($143 \pm 25 \text{ mg}\cdot\text{day}^{-1}$; range = 104 to 183 $\text{mg}\cdot\text{day}^{-1}$; 95% CI: 130.1 – 155.9; $n = 12$), and high consumers ($351 \pm$
63 $139 \text{ mg}\cdot\text{day}^{-1}$; range = 190 to 583 $\text{mg}\cdot\text{day}^{-1}$; 95% CI: 285.2 – 416.8; $n = 14$) (Table 1).

64

65 *Maximum oxygen consumption and workload capacity*

66

67 Participants performed a graded exercise test on a cycle ergometer (Lode Excalibur, Groningen, The
68 Netherlands) to determine $\text{VO}_{2\text{max}}$. Individual seat and handlebar position were recorded and replicated for each
69 subsequent visit. Participants began pedaling at a power output of 75 W, with 50 W increments every 4 min for
70 four submaximal stages. Thereafter, work rate was increased by 30 W every minute until volitional exhaustion.
71 The last completed stage plus the fraction of time spent in the final non-completed stage multiplied by 30 W
72 was defined as an individual's power max (W_{max}). Heart rate (HR) was monitored every minute using a
73 transmitter/telemetry unit (Polar System, Finland). Pulmonary gas exchange was determined breath by breath
74 for O_2 and CO_2 concentrations and minute ventilation by use of a portable gas analysis system (K4 b², Cosmed,
75 Rome, Italy). The gas analyzer was calibrated immediately before and verified after each test by using a
76 certified gravimetric gas mixture (BOC Gases, Chatswood, Australia). The ventilometer was calibrated pre-

77 exercise and verified post-exercise using a 3-liter syringe in accordance with the manufacturer's instructions.
78 There were no significant differences between pre- and post-test calibrations for any test.

79

80 *Simulated cycling time-trial*

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82 Participants reported to the laboratory at individually standardized times after a 6-h fasting period. Prior
83 to the main exercise, participants underwent a 5-min warm-up at 125 W, immediately followed by the
84 simulated cycling km TT, which has been shown to be reliable for recreationally-trained cyclists following a
85 familiarization session (CV = 2.9%, intraclass correlation = 0.87 [0.67 – 0.95], smallest meaningful change =
86 4.8 W; Oliveira et al., in press).(28) Participants were required to perform a set amount of work (mean: 420 ±
87 69 kJ) in the shortest time possible. Individual total work to be performed was calculated according to the
88 equation of Jeukendrup, Saris, Brouns, and Kester, 1996:(22)

89

$$90 \text{ Total amount of work} = 0.85 \times W_{\max} \times 1800$$

91

92 Where W_{\max} is the maximal workload capacity determined at day 1 and 1800 is duration in seconds
93 (equivalent to 30-min). The ergometer was set in linear mode so that 85% W_{\max} was obtained when the
94 participants cycled at 95 rpm. The participants were kept unaware of performance-related information (exercise
95 time and cadence) during the tests. The only information the participants received during the test was the
96 percentage of work performed relative to the preset task, namely following 25, 50, 75, 90 and 100% completion
97 of the total work done (%TWD). At these set intervals during the trial, participants were asked to rate their
98 perceived exertion (RPE) using the 6- to 20-point Borg scale(6) and a fingertip blood sample was collected for
99 the analysis of lactate. A small aliquot (20 μ L) of blood was taken and homogenized in a microtube containing
00 the same volume of an ice-cold 2% NaF solution. Samples were centrifuged at 2000 g for 5 min at 4°C to
01 separate plasma from erythrocytes. Plasma was removed and stored at -80 °C until analysis. Plasma lactate was
02 determined spectrophotometrically using an enzymatic-colorimetric method as supplied by a commercially
03 available kit (Katal, Intertec, Sao Paulo, Brazil).No encouragement was provided during the tests. Finally, all
04 persons not involved in the study were excluded from the laboratory to prevent any external disruption.

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Supplementation protocol

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Caffeine and food intake assessments

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Statistics

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The participants received a dose of anhydrous caffeine (6 mg·kg⁻¹BM) or placebo (dextrose) provided in gelatin capsules identical in color, size and appearance. No substance was administered in CON. The capsules were ingested with *ad libitum* water intake 60-min before the exercise tests. Participants rested quietly for 60-min before starting the test in all session. Supplements for each participant were prepared and separated by a non-affiliated researcher to ensure double-blinding.

Habitual caffeine intake was assessed by a specific FFQ adapted from a previously validated questionnaire(7) under the supervision of a qualified nutritionist. The questionnaire was employed to assess the habitual consumption of dietary products rich in caffeine. Portions, in household measures, were used to assess the amount of food consumed according to the following frequency of consumption: a) more than three times a day, b) two to three times a day, c) once a day, d) five to six times a week, e) two to four times per week, f) once a week, g) three times per month, h) rarely or never. The list was composed of 10 dietary products with high caffeine content, according to the Food and Drug Administration(32), namely: a) espresso and espresso drinks, b) brewed coffee, c) instant coffee, d) green tea, e) black tea, f) energetic drinks, g) regular and diet colas drinks, h) dark and milk chocolates, i) sweet cocoa powder, and j) caffeine supplements. Where possible, exact brands of products consumed were identified and subsequent caffeine content of these products obtained. Previously published nutritional tables were used for database construction.(8, 32) Food intake was assessed by means of six 24-h dietary recalls recorded before the exercise sessions (one VO_{2max} test, two familiarization sessions and three main trials). Energy and macronutrient intake were analyzed by the software Avanutri online (Avanutri, Rio de Janeiro, Brazil).

33 Data are presented as mean \pm standard deviation (with 95% confidence intervals - CI) and were analysed
34 using SAS statistical software (v. 9.3). Statistical significance was accepted at $P \leq 0.05$. Mixed-model analyses
35 with repeated measures were used to compare the overall effect of caffeine on time-trial performance (TT
36 performance) as well as to compare the food consumption between experimental trials; Trial (CON, PLA and
37 CAF) was used as a fixed factor and participants as a random factor. A mixed-model was used to analyse the
38 overall effect of caffeine on RPE and lactate, where Trial (CON, PLA and CAF) and %TWD (0, 25, 50, 75, 90
39 and 100%) were considered fixed factors.

40 The absolute changes in TT performance were also analysed using a mixed-model analysis of
41 covariance (ANCOVA) assuming habitual caffeine intake as a covariate, Trial (CAF and PLA) as a fixed factor
42 and participants as a random factor. A further mixed model was employed to examine the influence of habitual
43 caffeine intake (i.e., Consumption) on the absolute changes in TT performance (i.e., CAF – CON and PLA –
44 CON) in response to supplementation, where Consumption (low, moderate and high) and Trial (PLA and CAF)
45 were fixed factors. A Kenward-Roger correction was used to account for this unbalanced design and make
46 approximate inferences about these fixed effects in a mixed linear model. Tukey-Kramer adjustments for
47 multiple comparisons were performed whenever a significant F-value was obtained. The abovementioned
48 inferential analyses were also performed having the subjects divided into tertiles according to their habitual
49 caffeine intake relative to body weight. However, we opted for not reporting these data as i) results were
50 virtually the same despite the correction for body weight; and ii) literature commonly presents habitual caffeine
51 intake as absolute values,(5, 10) thus facilitating the contrast of findings.

52 Pearson's correlation was performed between habitual caffeine intake and the absolute change in TT
53 performance. Magnitude based inferences (MBIs) were used to determine the practical significance of caffeine
54 on TT performance using a spreadsheet to establish the likelihood of a meaningful effect on exercise
55 capacity.(3) The smallest worthwhile improvement in time to completion was 36 s, equivalent to half the
56 unbiased typical error associated with the measurement based upon the reliability data from 50 individuals.
57 Qualitative descriptors were assigned to the positive percentile scores as follows: <1%, *almost certainly not*
58 *beneficial*; 1-5%, *very unlikely beneficial*; 5-25%, *unlikely beneficial*; 25-75%, *possibly beneficial*; 75-95%,
59 *likely beneficial*; 95-99%, *very likely beneficial*; > 99%, *almost certainly beneficial*.(19) A Fischer Exact test

60 was used to determine any differences in i) supplement identification between trials, and ii) the proportion of
61 individuals who improved in CAF between tertiles.

62

63 RESULTS

64

65 *Overall effect of caffeine on TT performance, RPE and plasma lactate concentrations*

66 Three outliers were visually identified using boxplot analysis; each of these individuals had a
67 performance change in excess of 16% in CAF (two improved, one worsened). Thus, we performed all analyses
68 both with, and without, these three participants. Since the main outcomes remained the same, we decided to
69 only report the analyses with all individuals.

70 No order effect was shown ($F = 0.781$; $p = 0.461$). There was a main effect of Trial on TT performance
71 ($F = 9.472$; $P = <0.01$) (Figure 1A), with *post-hoc* tests revealing that performance in CAF was significantly
72 improved compared to PLA ($+2.4 \pm 5.5\%$; $P = 0.01$; 95% CI = -93.3 to -3.3 s; 78% likely beneficial) and CON
73 ($+3.3 \pm 6.4\%$; $P = <0.008$; 95% CI = -111.4 to -21.3 s; 92% likely beneficial). No significant difference in
74 performance was shown between PLA and CON ($+0.8 \pm 6.3\%$; $P = 0.62$; 95% CI = -63.1 to 26.9 s; 18%
75 unlikely beneficial).

76 There was no main effect of Trial ($F = 0.22$; $P = 0.805$) on RPE, but there was a significant main effect
77 of %TWD ($F = 118.08$; $P < 0.0001$); RPE increased throughout the exercise test although there were no
78 differences at any time point between CAF, PLA and CON (Figure 1B).

79 No main effect of Trial ($F = 1.44$; $P = 0.240$) on plasma lactate concentrations was observed, although
80 there was a significant main effect of %TWD ($F = 69.42$; $P < 0.0001$); plasma lactate significantly increased
81 with exercise compared to rest and post-ingestion for CAF, PLA and CON, although no differences at any time
82 point were observed between the trials. Nevertheless, a significant within-group increase was observed for CAF
83 at 100% compared to the previous time points (i.e., 0, 25, 50, 75, and 90%; all $P < 0.05$) (Figure 1C).

84

85

86 *Influence of habitual caffeine intake on TT performance, RPE and plasma lactate concentrations in*
87 *response to acute caffeine supplementation*

89 ANCOVA analysis showed no significant effect of habitual caffeine intake as a covariate ($F = 0.54$, $P =$
90 0.47). There was no Consumption x Trial interaction effect on absolute TT performance ($F = 0.47$; $P = 0.756$)
91 (Figure 2A), or on TT performance when CAF and PLA data were treated as absolute change compared to
92 CON ($F = 1.64$; $P = 0.207$). The absolute difference between CAF and CON was not significantly different
93 between tertiles (low vs moderate: $P = 1.000$; low vs high: $P = 0.996$; moderate vs high: $P = 0.986$) (Figure
94 2B), nor was the difference between CAF and PLA (data not shown).

95 MBIs suggested *possible to likely* improvements in CAF in the tertiles (Table 2). Individual analysis
96 showed that eight, seven and five individuals improved above the variation of the test with CAF compared to
97 CON in low, moderate and high, respectively (Figure 3), and a Fisher's Exact Test did not show any significant
98 differences in the number of individuals who improved between the tertiles ($P > 0.05$). There was no
99 correlation between habitual caffeine intake and absolute difference (i.e., CAF – CON) in performance (Figure
00 4).

01 No main effect of Consumption ($F = 0.63$; $P = 0.538$) or interaction effects (Consumption x Trial x
02 %TWD - $F = 0.58$; $P = 0.945$) were observed for RPE between tertiles (data not shown). In addition, no main
03 effect of Consumption ($F = 0.77$; $P = 0.470$) or interaction effects (Consumption x Trial x %TWD - $F = 1.02$; P
04 $= 0.437$) were detected for plasma lactate concentrations (data not shown).

05

06 *Food consumption analysis*

07

08 As expected, tertiles differed significantly in their habitual caffeine intake ($P = 0.0001$) (Table 1). No
09 significant differences for protein, carbohydrate, fat or caloric intake were observed between low, moderate and
10 high caffeine consumers before CAF, PLA and CON (Table 3).

11

12 *Side effects and blinding efficacy*

13

14 Tachycardia was the most frequently reported side effect in CAF ($n = 9$), followed by feelings of
15 increased wakefulness and attention ($n = 7$). Despite this, only 17 participants correctly guessed the supplement
16 ingested during both CAF and PLA. Moreover, 13 and 17 participants did not know what supplement they had

17 ingested during CAF and PLA. In addition, 12 and 8 participants incorrectly guessed the supplement ingested
18 during CAF and PLA. Fisher's Exact Test did not show any significant differences between trials for the
19 proportion of supplement identification ($P = 0.57$). Fisher's Exact Test did not demonstrate any significant
20 differences between low, moderate and high consumers for the reported side effects ($P = 0.77$).

21

22 **DISCUSSION**

23

24 There has been a long-standing paradigm that habitual caffeine intake may influence the ergogenicity of
25 caffeine supplementation. In contrast to this belief, this study showed that the level of habitual caffeine
26 ingestion was not associated with the magnitude of improvement in cycling TT performance following acute
27 caffeine supplementation.

28 Caffeine supplementation improved exercise performance by 3.3% compared to CON and 2.4%
29 compared to PLA. These data are in accordance with previous research, with meta-analytic data showing a
30 mean overall improvement of 1.9% with caffeine.(11) However, individual responses to caffeine
31 supplementation exist;(1) in our study, approximately half of the individuals improved above the variation of
32 the test with caffeine. Habitual caffeine intake has been suggested as a potential factor underlying
33 heterogeneous responses to this supplement.(5)

34 It is known that caffeine acts on the central nervous system antagonizing the adenosine A₁ and A₂
35 receptors, increasing circulating dopamine and noradrenaline.(27) Experimental data suggest that habitual
36 intake of caffeine is associated with an increased regulation of the adenosine receptors.(12) In support of this
37 notion, clinical studies have demonstrated a differential response to caffeine supplementation regarding mood
38 and cognitive performance in low and high caffeine consumers.(2, 18) This suggests that habitual caffeine
39 intake could influence exercise performance following caffeine supplementation, though current evidence is
40 contrasting.(5, 10) Recently, Beaumont, et al.(4) showed that 4 weeks of caffeine supplementation induced a
41 tolerance to the performance benefits of acute caffeine supplementation in low habitual caffeine consumers ($<$
42 $75 \text{ mg}\cdot\text{day}^{-1}$). However, the lack of a post-supplementation placebo trial, to provide a direct comparison with
43 the post-supplementation caffeine trial does not allow conclusive interpretation of the results of this study.
44 However, the relatively acute supplementation period (28 days) may have resulted in several acute adaptations

45 which do not represent chronic or habitual consumption. In the current study, there was no association between
46 habitual caffeine intake and exercise improvements with caffeine supplementation. Furthermore, low, moderate
47 and high consumers showed similar absolute and relative improvements in exercise performance. This concurs
48 with previous findings suggesting no difference between high and low consumers concerning caffeine's ability
49 to reduce muscle pain during cycling exercise, in which perceptions of pain and effort have been recognized as
50 part of pacing strategies.(14)

51 Divergent results seen in the literature could be partially explained by methodological differences.
52 Firstly, previous studies employed capacity tests which are known to have limited external validity and high
53 variability (>10%).(9, 20) We employed an exercise performance test with low variability (2.9%) in similarly
54 trained participants.(28) Additionally, we allocated participants into tertiles according to their habitual caffeine
55 intake as assessed by a validated FFQ method, resulting in distinct sub-groups with heterogeneous caffeine
56 intake (i.e., low: 2 to 101; moderate: 104 to 183; high: 190 to 583 mg·day⁻¹), allowing the investigation of a
57 broad spectrum of habitual caffeine intake on exercise performance in response to caffeine supplementation.
58 The calculated tertiles in this study are in agreement with the estimated average consumption of caffeine among
59 the healthy population (mean: 165 mg·day⁻¹; 90th percentile: 380 mg·day⁻¹).(26) In contrast, other studies
60 defined participants as caffeine consumers and naïve or non-consumers, without describing a method to support
61 this approach of categorizing individuals on opposite ends of the spectrum (i.e., <50 or >300 mg·day⁻¹).(5, 10)
62 The widely differing range used to characterize habitual caffeine intake may have contributed to previous
63 contrasting results. It also important to note that such a comprehensive comparison performed in the current
64 study was only possible due to the large sample size ($n = 40$), which is higher than previous studies (17 – 21
65 participants).(5, 10) In fact, this large sample allowed us to further explore our hypothesis *a posteriori* by
66 allocating participants into quartiles and quintiles, with still no significant differences between them in relation
67 to performance (data not shown).

68 Athletes are commonly encouraged to refrain from caffeinated products for up to 4 days before
69 supplementing with caffeine to enhance the efficacy of acute supplementation.(31) Despite this, Irwin, et al.(21)
70 showed similar improvements in exercise with caffeine in habitual consumers regardless of a 4 day withdrawal
71 period. Similarly, Van Soeren, et al.(35) showed equal exercise improvements with acute caffeine
72 supplementation in habituated consumers following no, 2-days and 4-days of caffeine withdrawal. In the

73 current study, to control the protocol, individuals were asked to refrain from caffeine only in the 24-h prior to
74 exercise. It could be suggested that this period of withdrawal may have created an artefact by “normalising” the
75 recent caffeine history of the participants. However, sudden caffeine cessation is unlikely to lead to withdrawal
76 symptoms, any of which are likely only to be moderate,(27) while the previously mentioned studies showed
77 that up to 4 days of caffeine withdrawal do not influence the exercise responses to an acute dose of caffeine
78 supplementation in habitual caffeine consumers.(21, 35) Therefore, we are satisfied that our methodology was
79 sufficient to test our initial hypothesis, although future research investigating the effects of habitual
80 consumption should perhaps record, and not prohibit, caffeine consumption in the 24 h preceding exercise.

81 At doses of 3-9 mg·kg⁻¹BM, caffeine is known to induce specific side effects, such as tachycardia,
82 anxiety, gastrointestinal discomfort, tremors, and insomnia.(17) It is believed that habitual caffeine consumers
83 are less susceptible to these side-effects at the same relative doses when compared to non-consumers.(15)
84 However, when participants were asked about the perceived effects 60-min after acute supplementation, they
85 reported the same side effects regardless of habitual caffeine intake. These results reinforce the notion that the
86 perceptual and exercise individual responses to caffeine may be triggered by other factors, such as genetics,
87 rather than habitual caffeine intake *per se*. In fact, genome-wide association studies have suggested that single
88 nucleotide polymorphisms in genes related to caffeine metabolism (aryl-hydrocarbon receptor [*AHR*],
89 cytochrome P450 1A1 and 1A2 [*CYP1A1-CYP1A2*, Prenyl (Decaprenyl) Diphosphate Synthase, Subunit 2
90 associated with habitual caffeine and coffee consumption.(23, 29) If the grouping of individuals in the current
91 study reflect different polymorphisms of various caffeine-related genes, then our data would challenge the
92 notion that certain genotypes result in carry over effects to performance. However, this is highly speculative
93 since genotypic analysis was beyond the scope of this study though further studies assessing caffeine-related
94 polymorphisms and exercise are warranted.

95 A limitation of this study is that we were unable to measure blood caffeine concentrations. However, all
96 previous studies employing doses of 3-9 mg·kg⁻¹BM 60-min before a given exercise task have reported
97 significant increases in caffeine concentration.(17, 34) Therefore, it can be assumed that our supplementation
98 protocol was effective in increasing blood caffeine levels. Furthermore, these findings must be confined to male
99 participants, since sexual dimorphism seems to exist in response.(33) We ensured tight control of our
00 experimental measures by having individuals attend the laboratory following a 6-h fast and 24-h post-caffeine

01 (and alcohol) ingestion. We acknowledge, however, that exercising in a fasted state does not represent the
02 recommendations of current sports nutrition guidelines or the “real life” practices of cyclists, nor does a
03 withdrawal period appear necessary to elicit performance improvements following acute caffeine
04 supplementation.(21) Although a $6 \text{ mg}\cdot\text{kg}^{-1}\text{BM}$ was employed in this study, we acknowledge that $3 \text{ mg}\cdot\text{kg}^{-1}\text{BM}$
05 is sufficient to induce performance effects.(17)

06 In conclusion, performance benefits with acute caffeine supplementation during a ~30 min cycling TT
07 were not influenced by the level of habitual caffeine consumption, refuting the long-standing notion that
08 habitual caffeine intake may negatively affect exercise performance in response to caffeine supplementation.

09

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21 **CONFLICT OF INTEREST**

22 The authors declare that they have no conflict of interest.

23

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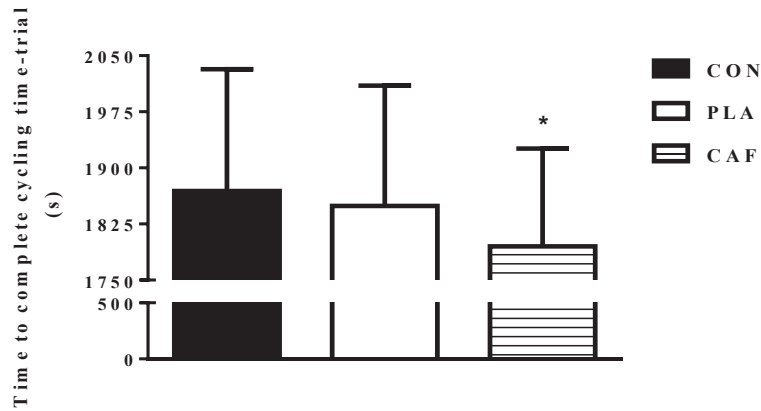
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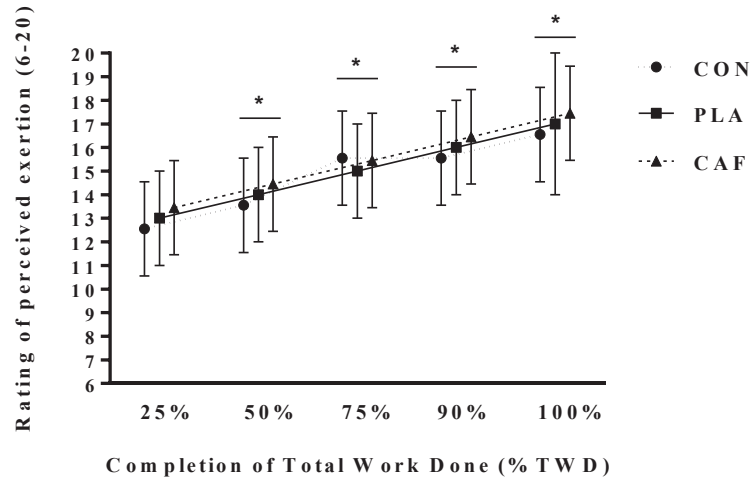
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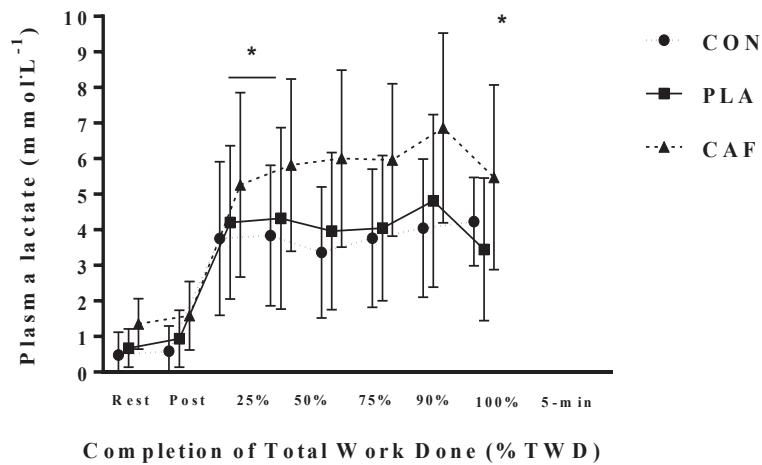


Figure 1: Panel A: Time-trial performance with caffeine (CAF), placebo (PLA) or no supplement (CON). * indicates significant difference ($P < 0.05$) compared to PLA and CON. Panel B: Rating of perceived exertion throughout the simulated time-trial with caffeine (CAF), placebo (PLA) or no supplement (CON). * indicates significant difference ($P < 0.05$) compared to the previous stage. Panel C: Plasma lactate ($\text{mmol}\cdot\text{L}^{-1}$) throughout exercise with caffeine (CAF), placebo (PLA) or no supplement (CON). * indicates significant difference ($P < 0.05$) compared to the previous stage.

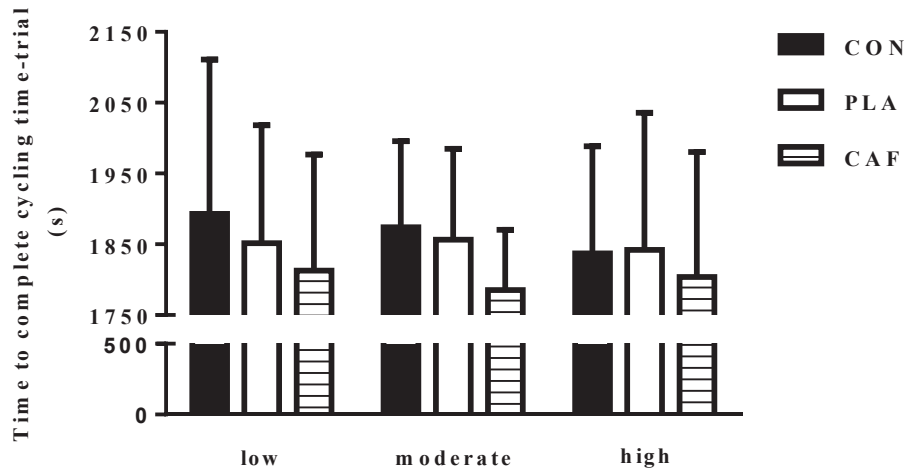
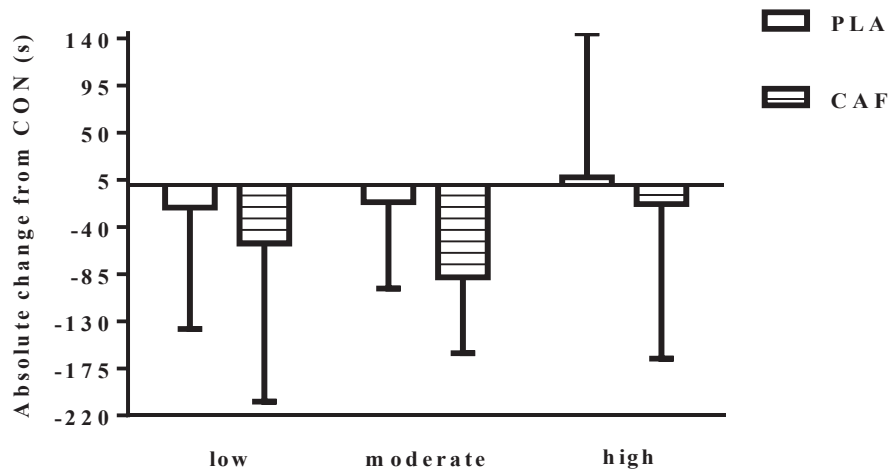
A**B**

Figure 2: Panel A: Time-trial performance in caffeine (CAF), placebo (PLA) or no supplement (CON) separated according to habitual caffeine intake ($P > 0.05$). Panel B: Absolute change (compared to CON) in time-trial performance after caffeine (CAF) or placebo (PLA) separated according to habitual caffeine intake ($P > 0.05$).

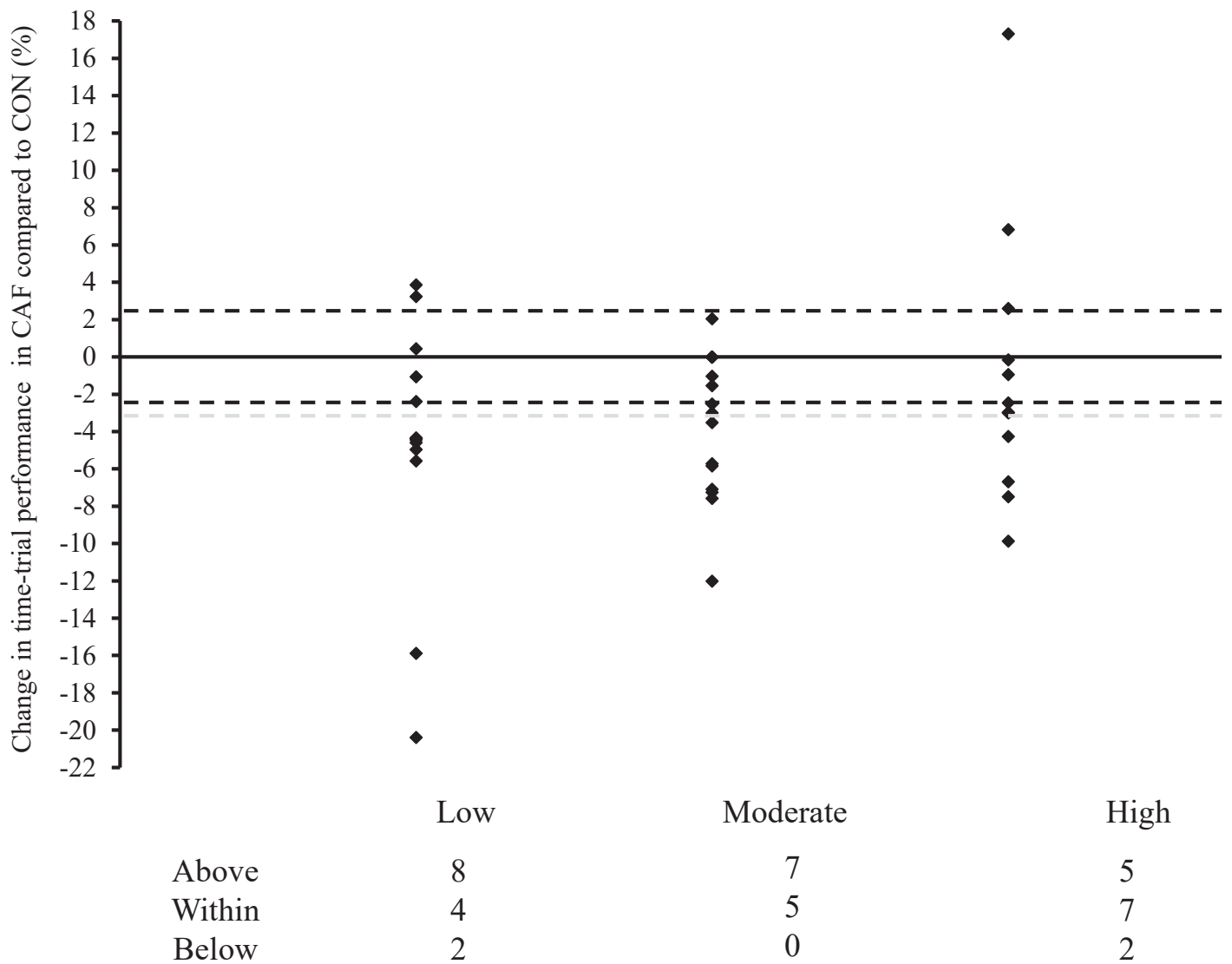


Figure 3: Individual relative change in time-trial performance in CAF vs. CON for low, moderate and high consumers. The black dashed line represents the natural variation of the test ($\pm 3.0\%$); the grey dashed line represents the mean overall improvement with caffeine ($+3.3\%$). The number of individuals who improved above, were within, or worsened beyond the natural variation of the test in each sub-group is displayed below the graph ($P > 0.05$).

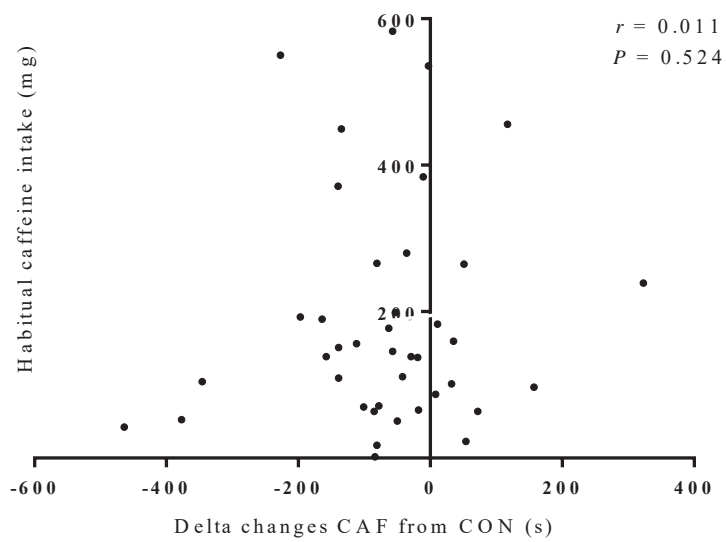


Figure 4: Correlation between habitual caffeine intake and absolute change in exercise performance in CAF vs. CON ($P > 0.05$).

Table 1. Participant characteristics

	Low	Moderate	High
Mean (SD)	(<i>n</i> =14)	(<i>n</i> =12)	(<i>n</i> =14)
Age	34 ± 9	37 ± 7	37 ± 8
Height (m)	1.74 ± 0.08	1.77 ± 0.05	1.77 ± 0.03
Weight (kg)	72.08 ± 11.13	74.95 ± 4.92	76.05 ± 8.42
VO _{2max} (ml·kg ⁻¹ ·min ⁻¹)	50.10 ± 8.45	51.00 ± 6.08	50.90 ± 7.54
Caffeine intake (mg/day)*	58 ± 29	143 ± 25	351 ± 139

Low, moderate and high = habitual caffeine intake

*Significant difference between tertiles (*P* < 0.05)

Table 2. Magnitude-based inferences for time-trial performance across tertiles

Difference (%)	Chances of treatment	Chances of treatment	Chances of treatment being
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		being positive (%)	being trivial (%)	negative (%)
Low				
CAF vs CON	-3.71 ± 7.62	82	17	1
CAF vs. PLA	-1.57 ± 4.25	51	49	0
PLA vs. CON	1.50 ± 15.25	53	44	3
Moderate				
CAF vs CON	-4.57 ± 3.63	99	1	0
CAF vs. PLA	-3.69 ± 4.65	93	6	1
PLA vs. CON	-0.28 ± 9.83	40	53	7
High				
CAF vs. CON	-0.88 ± 7.85	49	46	5
CAF vs. PLA	-1.4 ± 7.68	55	43	2
PLA vs. CON	-1,38 ± 13,77	14	66	20

Legend: CON = control, PLA = placebo; CAF = caffeine
 Low, moderate and high = habitual caffeine intake

Table 3: Food consumption data of participants **prior to the experimental sessions.**

	CON			PLA			CAF		
	Low	Moderate	High	Low	Moderate	High	Low	Moderate	High
Protein (g)	114 ± 42	114 ± 36	123 ± 46	122 ± 61	99 ± 37	105 ± 59	114 ± 59	104 ± 64	128 ± 58
Carbohydrate (g)	360 ± 89	347 ± 105	334 ± 144	354 ± 108	295 ± 76	306 ± 164	357 ± 132	351 ± 151	319 ± 222
Fat (g)	82 ± 21	80 ± 29	77 ± 22	87 ± 36	66 ± 25	80 ± 46	86 ± 30	74 ± 46	84 ± 28
Caloric intake (kcal)	2548 ± 553	2711 ± 1116	2631 ± 1045	2708 ± 716	2338 ± 504	2383 ± 1032	2637 ± 877	2474 ± 1062	2628 ± 1051