

1 **Placebo in sports nutrition: a proof-of-principle study involving caffeine**
2 **supplementation**

3

4 Original article

5 Running head: Placebo in sports nutrition

6

7 Bryan Saunders¹, Luana Farias de Oliveira¹, Rafael Pires da Silva¹, Vitor de Salles
8 Painelli¹, Livia Souza Gonçalves¹, Guilherme Yamaguchi¹, Thiago Mutti¹, Erika
9 Maciel³, Hamilton Roschel^{1,2}, Guilherme Giannini Artioli^{1,2}, Bruno Gualano^{1,2}

10

11 ¹Applied Physiology & Nutrition Research Group, University of São Paulo, Brazil.

12 ² Rheumatology Division, School of Medicine, University of São Paulo, Brazil.

13

14 Correspondence:

15 Bruno Gualano

16 Av. Mello de Moraes 65

17 Butanta, 05508-030

18 Sao Paulo, SP, Brazil.

19 Phone: +55 11 3091-3096

20 Fax: +55 11 3813-5921

21 E-mail: gualano@usp.br

22

23 **Abstract**

24 We investigated the effects of supplement identification on exercise performance with
25 caffeine supplementation. Forty-two trained cyclists (age 37 ± 8 y, body mass [BM]
26 74.3 ± 8.4 kg, height 1.76 ± 0.06 m, maximum oxygen uptake 50.0 ± 6.8 ml·kg⁻¹·min⁻¹)
27 performed a ~30 min cycling time-trial 1 h following either 6 mg·kg⁻¹BM caffeine
28 (CAF) or placebo (PLA) supplementation and one control (CON) session without
29 supplementation. Participants identified which supplement they believed they had
30 ingested (“caffeine”, “placebo”, “don’t know”) pre- and post-exercise. Subsequently,
31 participants were allocated to subgroups for analysis according to their identifications.
32 Overall and subgroup analyses were performed using mixed-model and magnitude
33 based inference analyses. Caffeine improved performance vs. PLA and CON
34 ($P\leq 0.001$). Correct pre- and post-exercise identification of caffeine in CAF improved
35 exercise performance (+4.8 and +6.5%) vs. CON, with slightly greater relative
36 increases than the overall effect of caffeine (+4.1%). Performance was not different
37 between PLA and CON within subgroups (all $P>0.05$), although there was a tendency
38 towards improved performance when participants believed they had ingested caffeine
39 post-exercise ($P=0.06$; 87% *likely beneficial*). Participants who correctly identified
40 placebo in PLA showed *possible harmful effects* on performance compared to CON.
41 Supplement identification appeared to influence exercise outcome and may be a
42 source of bias in sports nutrition.

43 **Key words:** Placebo effect; nocebo effect; expectancy; exercise performance;
44 caffeine supplementation; supplement identification; cycling time-trial

45

46 **Introduction**

47 Contemporary investigations into the effects of nutritional interventions on exercise
48 generally employ double-blind and placebo controlled study designs to ensure there is
49 no bias from the prior knowledge of which substance has been ingested and that
50 comparisons can be made against an appropriate control. The placebo effect, namely a
51 positive outcome brought about purely from the belief that one has received a positive
52 intervention (Clark et al., 2000), can mask the true effect of an intervention. The
53 nocebo effect is directly opposite to this in that a negative outcome occurs following
54 the administration of an intervention (Benedetti et al., 2007; (Lundby et al., 2012;
55 Pollo et al., 2012).

56

57 Caffeine-based investigations can be difficult to blind due to the associated side-
58 effects at high doses (*i.e.*, $>2-3 \text{ mg}\cdot\text{kg}^{-1}\text{BM}$), namely tachycardia and agitation
59 (Graham & Spriet, 1995), and common knowledge thereof. Once an individual
60 believes that they have ingested a performance enhancing substance, several
61 behaviours may be modified that can contribute to exercise performance (Beedie et
62 al., 2006). This may lead to many of the participants beginning exercise with a greater
63 expectancy due to the occurrence of physiological side effects making it difficult to
64 separate the true effect of caffeine from its associated placebo effect. However, most
65 studies do not control whether blinding of the intervention was successful;
66 determination of an individual's belief of what they have ingested prior to exercise
67 may lead to further investigation into the effects of preconceptions (placebo effect) on
68 exercise.

69

70 In addition to preconceptions, it would be reasonable to suggest that any behavioural
71 processes that might have been modified prior to exercise might also change
72 throughout exercise on the basis of new information (Beedie et al., 2006). This might
73 relate to an individual's perceived effort throughout exercise, which may or may not
74 be influenced by the intervention itself. An individual who believed they had ingested
75 placebo prior to exercise but then changes opinion due to a good start may influence
76 their pacing accordingly throughout the test. Conversely, someone who expects to
77 improve performance due to preconceived opinion of ingesting the active substance,
78 but subsequently struggles to perform, might suffer a reduction in performance due to
79 a further lack of motivation. Therefore, it would also be of interest to determine the
80 individual's perception of what was ingested following exercise to determine whether
81 the initial opinion has been modified throughout the protocol.

82

83 Therefore, to advance the knowledge on the influence of the placebo effect in sports
84 nutrition, we investigated the effect of supplement identification following caffeine
85 ingestion on exercise performance. We hypothesised that caffeine supplementation
86 would improve exercise performance regardless of proper identification, and that
87 improvements would be greatest in those who correctly guessed they had taken
88 caffeine, while participants ingesting placebo but guessed they had ingested caffeine
89 would also improve their exercise performance.

90

91 **Materials and Methods**

92 *Participants*

93 Forty-two trained male cyclists (Table 1) volunteered and gave their written informed
94 consent to participate in this study. The exclusion criteria included the use of beta-
95 alanine and creatine in the past 6 months, the presence of any musculoskeletal
96 disorder, or the current or past use of anabolic steroids or other illicit performance-
97 enhancing drugs. Habitual caffeine consumption (Table 1) was assessed prior to
98 inclusion in the study via a Food Frequency Questionnaire adapted from two
99 previously developed and validated questionnaires (Bühler et al., 2014 and Fred
100 Hutchinson Cancer Research Center, 2004). Although these data were not used to
101 exclude any participant *per se*, any participant ingesting caffeine as a dietary
102 supplement was not included in the study since these individuals may or may not have
103 been more susceptible to correct supplement identification due to experience. The
104 study was approved by the University of São Paulo's Ethics Review Committee as
105 part of a larger thematic project, the remaining data of which is presented elsewhere.

106

107 *Experimental Design*

108 All participants attended the laboratory on six separate occasions following a
109 minimum 6-h fasting period. All trials were performed at the same time of day for
110 each participant (between 08:00 and 20:00) to ensure results were not affected by
111 circadian variation (Reilly and Brooks, 1986). All tests were performed on a cycle
112 ergometer (Lode Excalibur, Germany) and separated by a minimum of 72 h. The first
113 session comprised of an incremental cycling test to exhaustion to determine VO_{2max}
114 and maximal cycling output (W_{max}). In the remaining five sessions, participants
115 performed a simulated time trial, namely two familiarisation sessions and three main

116 trials (caffeine - CAF, placebo - PLA, and control - CON). Twenty-four hours prior to
117 the main trials, participants were required to refrain from alcohol, caffeine and any
118 unaccustomed strenuous exercise. Food intake was monitored during the 24-h period
119 prior to the main trials using a food diary. Food diaries were analysed by a nutritionist
120 immediately prior to the experimental sessions to ensure that participants had not
121 consumed any caffeine containing foods while energy and macronutrient intake was
122 analysed at a later time by the same nutritionist using specific software (Avanutri
123 online, Avanutri, Rio de Janeiro, Brazil).

124

125 Main trials were performed in a double-blind, randomised, counterbalance and cross-
126 over manner. For the CAF and PLA trials, participants ingested a capsule containing
127 either 6 mg·kg⁻¹BM of caffeine or dextrose alongside 500 mL of water. Participants
128 were then required to remain seated for 1 h prior to the commencement of the main
129 exercise protocol. During the CON trial, participants followed the same procedures
130 although they did not consume any capsule prior to exercise. Participants were
131 allowed access to their phones or own reading material throughout this waiting
132 period. Blinding occurred via an outside researcher who prepared each participant's
133 supplements in identical looking opaque capsules. Participants were randomly
134 assigned to each experimental condition using a Latin Square model (Mason et al.,
135 2003).

136

137 In each supplementation trial, participants were required to respond to a standardised
138 question immediately prior to exercise (*i.e.* 1 h post-supplement ingestion) and again
139 immediately following completion of the exercise. The question related to their belief
140 of which supplement they had taken and was given with the option of choosing one of

141 three possible answers (*i.e.* “Which supplement do you think you have ingested?” a)
142 Caffeine b) Placebo c) Don’t know). They were also asked to state the reason they had
143 chosen their answer (Supplementary Tables 1 and 2). Based upon each participant’s
144 answer, subgroups were composed according to the supplement trial (*i.e.* CAF or
145 PLA), supplement identification (*i.e.* “correct; “don’t know”; “wrong”), and the
146 moment in which the question was answered (*i.e.* Pre-exercise identification; Post-
147 exercise identification).

148

149 **Experimental Procedures**

150 *Incremental cycling capacity test*

151 Each participant performed a graded cycle capacity test to exhaustion on a cycle
152 ergometer (Lode Excalibur, Germany) to determine individual VO_{2max} and W_{max} .
153 Individual set up of the cycle ergometer (saddle and handlebar height and length) was
154 determined prior to the maximal test, recorded electronically and maintained for all
155 subsequent trials. Participants were required to perform four submaximal 4-min stages
156 starting at 75 W; this was increased by 50 W each stage until 225 W. Thereafter,
157 workload was increased by 30 W every minute until volitional exhaustion. Ventilatory
158 and gas exchange measurements were recorded using a portable breath-by-breath
159 system (K4 b², Cosmed, Italy) which has previously been validated (McLaughlin et
160 al., 2001); the highest value averaged over a 30-s period during the test was defined as
161 VO_{2max} . The last completed stage plus the fraction of time spent in the final non-
162 completed stage multiplied by 30 W was defined as a participant’s W_{max} .

163

164 *Cycling Time-Trial (TT)*

165 The cycling TT was performed on a cycle ergometer (Lode Excalibur, Germany).
166 Participants were required to perform a 5-min cycling warm up performed at 125 W
167 followed immediately by the TT. Participants performed the TT in which they were
168 required to complete a predetermined amount of work equivalent to 25 min at 85% of
169 their individual W_{\max} in the fastest possible time; this was based on the protocol of
170 Jeukendrup et al. (2008).

171 The formula for total amount of work to be performed was as follows:

$$172 \quad \text{Total amount of work} = 0.85 \times W_{\max} \times 1500 \text{ s}$$

173 The average amount of work to be completed for all participants was 420.3 ± 68.6 kJ.

174 The cycle ergometer was set in linear mode, meaning work load was cadence-
175 dependent according to the formula:

$$176 \quad W = \alpha \times (\text{rev} \cdot \text{min}^{-1})^2$$

177 The α value was based on each participant's W_{\max} so that they were working at 85%
178 W_{\max} when cycling at a cadence of $95 \text{ rev} \cdot \text{min}^{-1}$. Participants were instructed to
179 complete the exercise in the fastest possible time. No motivation or specific
180 information was given to the participants during the test although they were informed
181 when they had completed 25%, 50%, 75% and 90% of the exercise. Mean power
182 output (MPO, W) was recorded as the outcome measure for the TT. In order to
183 determine the reliability of the test, we conducted a further test-retest study on 50
184 participants who completed the TT on two occasions. There was no significant
185 difference in MPO between tests (227.2 ± 35.4 and 224.5 ± 34.7 W) with a coefficient
186 of variation of $3.0 \pm 2.3\%$.

187

188 *Statistical Analysis*

189 Exercise data (MPO) was compared by mixed model analysis in order to determine
190 the effect of supplementation on exercise. To ensure there was no learning effect, the
191 effect of trial order was determined with trial considered a fixed factor and
192 participants a random factor. For the overall analysis, supplementation was assumed
193 as a fixed factor and participants as a random factor. To investigate the effect of
194 expectation on exercise, further sub-analyses were performed according to pre- and
195 post-exercise responses to the questionnaire. Participants were grouped according to
196 their supplement identification (“correct”; “don’t know”; “wrong”) in CAF and PLA
197 and subsequent exercise data within these subgroups was compared to CON. Analyses
198 of these data were performed in an identical manner to the overall data, assuming
199 supplementation as a fixed factor and participant as a random factor. Tukey post-hoc
200 tests were performed whenever a significant F-value was obtained and the
201 significance level was previously set at $P \leq 0.05$. All these analyses were conducted
202 using SAS software (SAS® version 9.3, Cary, NC, USA) and are presented as mean \pm
203 1SD unless otherwise stated. Magnitude based inferences (MBI; Batterham and
204 Hopkins, 2006) were used to determine the practical significance of caffeine on TT
205 performance using a spreadsheet to establish the likelihood of a meaningful effect on
206 exercise capacity. The smallest worthwhile improvement in MPO was calculated
207 using half the CV of the test (Hopkins, 2004; Paton and Hopkins, 2006). Qualitative
208 descriptors were assigned to the positive percentile scores as follows: <1%, *almost*
209 *certainly not*; 1-5%, *very unlikely*; 5-25%, *unlikely*; 25-75%, *possibly*; 75-95%, *likely*;
210 95-99%, *very likely*; >99%, *almost certainly* (Hopkins, 2002). Additionally, the
211 estimated means and SDs from CAF and PLA, separated according to supplement
212 identification, were used to calculate Cohen’s *d* (Cohen, 1988) effect sizes and
213 confidence intervals (CI) to plot between-trial comparisons. It is important to note that

214 direct comparisons could not be made between the overall effects vs. the within
215 subgroup effects (e.g., “Overall CAF vs. CON” versus any sub-group within CAF)
216 since this would result in analysis of duplicate data (considering some of the
217 participants’ data in overall CAF and PLA are also included within their specific sub-
218 groups). Therefore, these comparisons and subsequent interpretation were based upon
219 MBIs, percentage and absolute changes, and individual responses.

220

221 **Results**

222 *Questionnaires*

223 *Pre-exercise identification*

224 In CAF, seventeen participants correctly identified caffeine, while twelve incorrectly
225 identified placebo with a further thirteen choosing “don’t know”. Seventeen
226 participants correctly identified placebo in PLA, eight believed they had ingested
227 caffeine, and the remaining seventeen chose “don’t know”.

228

229 *Post-exercise identification*

230 Twenty participants correctly identified the supplement following exercise in CAF,
231 while fourteen were incorrect and a further eight chose “don’t know”. Eighteen
232 participants correctly assumed that they had taken placebo in PLA, while eleven
233 believed they had ingested caffeine, and thirteen were unsure as to what they had
234 ingested choosing “don’t know”.

235

236 A total of thirteen and fourteen participants changed their supplement identification in
237 CAF and PLA from pre- to post-exercise. Six participants correctly identified caffeine
238 post-exercise having previously been incorrect (“placebo”, N = 3) or choosing “don’t
239 know” (N = 3). Three participants who had correctly identified caffeine changed their
240 mind to placebo (N = 1) or “don’t know” (N = 2) following exercise, while four
241 participants who chose “don’t know” prior to exercise incorrectly guessed that they
242 had ingested placebo. Six participants changed their previously unsure (“don’t know”,
243 N = 5) and incorrect (“caffeine”, N = 1) opinions to correctly identify placebo in PLA.
244 Five participants changed their opinion to “don’t know” (N = 2) and caffeine (N = 3)
245 having correctly identified placebo prior to exercise. Two participants who chose

246 “don’t know” pre-exercise, incorrectly identified caffeine at post-exercise and one
247 participant changed his pre-exercise identification of “caffeine” to “don’t know” at
248 post-exercise.

249

250 *Exercise results*

251 *Overall*

252 There was no effect of trial order on MPO ($P = 0.58$). There was an overall effect of
253 supplement on MPO ($P = 0.0002$) with *post hoc* analyses revealing an improved
254 performance in CAF vs. PLA ($+3.0 \pm 5.8\%$, 234.2 ± 36.7 vs. 228.0 ± 37.6 W, $P =$
255 0.007 ; 91% *likely beneficial*) and vs. CON ($+4.1 \pm 6.2\%$, 234.2 ± 36.7 vs. $225.7 \pm$
256 38.4 W, $P = 0.0002$; 99% *very likely beneficial*), but no difference between PLA and
257 CON ($P = 0.50$; 24% *unlikely beneficial*). Twenty-three participants improved above
258 the variation of the test in CAF and twelve in PLA.

259

260 *Pre-exercise identification*

261 Correct supplement identification in CAF resulted in improved MPO ($P \leq 0.001$;
262 100% *almost certainly beneficial*) compared to CON (Table 2). Similarly, incorrect
263 identification in CAF resulted in improved performance compared to CON ($P =$
264 0.003 ; 99% *very likely beneficial*), but there was no difference for participants who
265 chose “don’t know” ($P = 0.95$; 16% *unlikely beneficial*) (Table 2). Effect sizes and
266 CIs are presented in Figure 1. Eleven of the seventeen participants who correctly
267 identified caffeine improved above the variation of the test, while four of thirteen
268 were improved having chosen “don’t know” and eight of twelve having incorrectly
269 identified placebo (Figure 2).

270

271 There were no statistical differences in MPO between PLA and CON within
272 supplement identification subgroups (all $P > 0.05$; Table 2), although magnitude
273 based inferences suggested correct identification of “placebo” in PLA led to *possibly*
274 *harmful* effects on performance. Effect sizes and CIs are presented in Figure 1. Four
275 participants who correctly identified “placebo” showed performance reductions above
276 the variation of the test. Twelve participants improved above the variation of the test
277 in PLA; three who correctly identified placebo, five who chose “don’t know” and four
278 who believed they ingested caffeine (Figure 2).

279

280 *Post-exercise identification*

281 Participants who correctly identified caffeine in CAF improved MPO compared to
282 CON ($P \leq 0.001$; 100% *almost certainly beneficial*; Table 2) Participants who
283 incorrectly identified placebo in CAF also improved performance compared to CON
284 ($P = 0.03$; 90% *likely beneficial*; Table 2), but there was no difference in performance
285 in those who did not identify any supplement ($P > 0.05$; 58% *likely trivial*; Table 2).
286 Effect sizes and CIs are presented in Figure 1. Fifteen of the twenty participants who
287 correctly identified caffeine improved above the variation of the test, while seven of
288 fourteen improved despite incorrectly identifying placebo. Of the eight who chose
289 “don’t know”, only one improved performance (Figure 2).

290

291 Performance was not statistically different between PLA and CON for participants
292 who chose “don’t know” ($P > 0.05$; Table 2). There was a tendency towards improved
293 MPO ($+3.7 \pm 6.3\%$, $P = 0.06$; 87% *likely beneficial*) in those who incorrectly believed
294 they had ingested caffeine in PLA (Table 2), while MBIs suggested a *possibly*
295 *harmful* effect of correct identification of placebo ($-1.6 \pm 4.9\%$) and only a 1% chance

296 of being positive. Effect sizes and CIs are presented in Figure 1. Six participants
297 improved above the variation having incorrectly identified caffeine, while five
298 improved having chosen “don’t know”. Only one participant improved having
299 correctly identified placebo while six worsened performance (Figure 2).

300

301 *Food intake*

302 Absolute and relative carbohydrate, protein, and fat intake in the 24 h prior to the
303 main trials were not significantly different (all $P > 0.05$). Similarly, total caloric
304 intake was not different prior to any trial ($P = 0.93$).

305 **Discussion**

306 This study showed that correct identification of caffeine, particularly post-exercise,
307 improved cycling performance with greater relative improvements than the overall
308 effect of caffeine. Furthermore, there was an apparent improved performance in PLA
309 for participants who believed they had ingested caffeine, although this was based
310 upon post-exercise supplement identification only, while correct identification of
311 placebo, both pre- and post-exercise, may possibly have led to performance
312 impairments.

313

314 This study employed trained cyclists, the majority of whom were competing at
315 national and international level. Although none took caffeine as a supplement, all
316 participants were aware of the substance and its purported ergogenic effect. Thus, it is
317 reasonable to suggest that any individual who identified the supplement ingested as
318 caffeine will have had the belief that their performance would improve accordingly.
319 Indeed, correct identification of caffeine ingestion resulted in an improved
320 performance with greater relative improvements than the overall effect of caffeine
321 (Pre-exercise: +4.8% and Post-exercise: +6.5% vs. Overall: +4.1%; Figure 2). The
322 questionnaire allowed an uncertainty regarding which supplement had been ingested
323 (“don’t know”). Thus, analysing participants who chose this response would
324 theoretically allow determination of the “true effect” of caffeine since the individual
325 would not be biased by opinion. Surprisingly, however, performance was unaffected
326 with caffeine when participants were unsure as to what they had ingested, but was
327 improved when they incorrectly identified placebo (Table 2). We can only speculate
328 as to the reason for these unexpected findings; perhaps the physiological mechanisms
329 by which caffeine improves performance were a greater stimulus in participants

330 believing they had ingested a placebo substance, or there may have been an increased
331 motivation in these participants. Nonetheless, this was not directly measured here
332 though future investigation should consider this.

333

334 Interestingly, post-exercise identification of caffeine in PLA showed a tendency
335 towards improved performance despite participants having ingested no active
336 substance. Increases in this subgroup were *likely beneficial*, above the variation of the
337 test (+3.7 vs. +3.0%) and very close to the overall beneficial effect of caffeine shown
338 in the current study (~4.0%). Beedie et al. (2006) previously investigated the effects
339 of expectation on performance; participants were informed that they had ingested
340 either 4.5 or 9.0 mg·kg⁻¹BM prior to exercise although caffeine was not administered
341 on any occasion. Despite this, the authors showed a *likely beneficial* 2.2% in 10 km
342 TT performance when participants believed they had ingested caffeine, which is
343 similar to the performance increase of ~3.5% according to post-exercise caffeine
344 identification in PLA in the current study. Taken together, these results support the
345 notion that the belief that one has ingested an active supplement can strongly
346 influence the outcome of an exercise task (Clark et al., 2000). Furthermore, it seems
347 reasonable to speculate that expectation, which is highly variable among individuals,
348 is a factor that can potentially account for some of the variability in responses to
349 certain interventions in sports nutrition.

350

351 Indeed, it is apparent that correct identification of placebo may have impeded
352 performance with *possibly harmful* effects and a total of four (pre-exercise
353 identification) and six (post-exercise identification) participants worsening
354 performance beyond the variation of the test. The nocebo effect is directly opposite to

355 the placebo effect in that a negative outcome occurs following the administration of
356 an inert intervention (Benedetti et al., 2007). This phenomenon has been shown to
357 reduce exercise performance (Lundby et al., 2012; Pollo et al., 2012) and increase
358 ratings of perceived exertion (Bottoms et al., 2014), but it has been rarely addressed
359 scientifically, particularly in sports nutrition. Interestingly, based upon our findings, it
360 appears that correct identification of placebo by some athletes expecting to receive a
361 potential ergogenic aid may result in the nocebo effect, possibly by frustrating their
362 expectations. However, the opposite appeared true in individuals who believed they
363 had ingested placebo when taking caffeine. While it remains unclear as to why and
364 how active and non-active substances can differently modulate expectations and
365 performance, this study provides some evidence to suggest that the nocebo effect may
366 play a role in performance outcomes and should be accounted for within any
367 experimental investigation or clinical intervention in sports nutrition.

368

369 Correct (+4.8%) and incorrect (+7.3%) pre-exercise supplement identification in CAF
370 resulted in performance improvements above the overall effect (+4.1%). Post-
371 exercise, incorrect placebo identification fell below this overall improvement (+3.3%)
372 while correct identification of caffeine improved further (+6.5%). These changes are
373 due to a number of participants changing their opinion from pre- to post-exercise,
374 likely due to stimuli relating to the exercise (Beedie et al., 2006). The majority of the
375 stated reasons for believing caffeine had been ingested prior to exercise were due to
376 the sensation of caffeine associated side effects, specifically tachycardia, alertness and
377 trembling. Additionally, a number of participants' reasons for identifying caffeine
378 post-exercise appear to be due to stimuli felt throughout the exercise test, namely
379 "feeling better" or "less tired". This was particular true with respect to the eleven

380 individuals who changed their opinion to caffeine, six of whom (four in CAF; two in
381 PLA) improved their performance above the variation of the test. Thus, it could be
382 suggested that post-exercise supplement identification may be the most accurate
383 measurement relating to perception since it incorporates both conceptions prior to
384 (*i.e.*, side-effects) and during (*i.e.*, side-effects and performance effects) the exercise.
385 However, the main limitation of this study is that we did not determine why
386 participants changed their opinion. Furthermore, it cannot be fully elucidated whether
387 any participant's change in supplement identification resulted from their performance
388 or whether it shaped the performance itself. Nonetheless, these data support the notion
389 that preconceptions may be further modified by factors intrinsic to exercise (Beedie et
390 al., 2006), and thus should be taken into account. Future research should include pre-
391 and post-exercise questionnaires including the opportunity to discuss why opinions
392 were modified.

393

394 The results of this study highlight the necessity in assessing a participants' perception
395 of what they have ingested in order to distinguish the true effect of a supplement from
396 its placebo effect. Importantly, simply including a placebo group may not be
397 sufficient to effectively blind an experiment; active nutrients and drugs, such as
398 caffeine, beta-alanine, sodium bicarbonate and creatine, may cause side effects or
399 changes in performance, which are clues leading subjects to identify the treatment. To
400 avoid bias in the analysis of results, it would be prudent to test the efficacy of the
401 blinding procedure by asking participants to identify the supplement ingested.
402 Comprehensive assessment of data according to perceptions of the supplement
403 ingested could allow for more definitive conclusions on the actual effects of active
404 nutrients in sports nutrition. In contrast to the undesirable effect of preconception in

405 research, any such bias may prove positive in a real world setting. It would be
406 reasonable to suggest that an athlete may benefit solely from the belief that he has
407 ingested an active supplement, a notion previously suggested to have some scientific
408 basis (de la Fuente-Fernandez et al., 2002; Yang et al., 2002).

409

410 **Perspective**

411 Correct identification of caffeine, particularly after exercise, appeared to improve
412 cycling performance to a greater extent than the overall effect of caffeine.
413 Furthermore, participants who believed they had ingested caffeine while ingesting
414 placebo also appeared to improve their performance while correct identification of
415 placebo may lead to possible impairments in performance for some individuals.
416 Altogether, these results suggest that an individual's perception of whether they have
417 ingested an active supplement contributes greatly to their exercise performance,
418 although the mechanisms by which this influences performance remain to be fully
419 elucidated. Scientists must be encouraged to systematically test whether their blinding
420 procedure was effective when interpreting data as this is likely a source of bias in
421 sports nutrition.

422

423 **Acknowledgements**

424 Bryan Saunders, Vitor de Salles Painelli, Guilherme Giannini Artioli and Bruno
425 Gualano have been financially supported by Fundação de Amparo à Pesquisa do
426 Estado de Sao Paulo (FAPESP grants number: 2011/19513-2, 2013/04806-0,
427 2014/11948-8 and 2013/14746-4). Bryan Saunders (150513/2015-1), Bruno Gualano
428 and Hamiton Roschel have been financially supported by Conselho Nacional de
429 Desenvolvimento Científico e Tecnológico (CNPq).

430 We wish to thank the Laboratório de Determinantes Energéticos de Desempenho
431 Esportivo (LADESP) for access to the cycle ergometer used in this study and the
432 volunteers for their efforts.

433

434 **Conflict of interest**

435 The authors declare that they do not have conflict of interests.

436

437 **References**

438

- 439 1. Batterham AM, Hopkins WG. Making meaningful inferences about
440 magnitudes. *Int J Sports Physiol Perf* 2006; 1(1): 50-57.
- 441 2. Beedie CJ, Foad AJ. The placebo effect in sports performance. *Sports Med*
442 2009; 39(4): 313-329.
- 443 3. Beedie CJ, Stuart EM, Coleman DA, Foad AJ. Placebo effects of caffeine on
444 cycling performance. *Med Sci Sports Exerc* 2006; 38(12): 2159-2164.
- 445 4. Benedetti F, Amanzio M, Vighetti S, Asteggiano G. The biochemical and
446 neuroendocrine bases of the hyperalgesic nocebo effect. *J Neurosci* 2006:
447 26(46): 12014-1202
- 448 5. Bottoms L, Buscombe R, Nicholettos A. The placebo and nocebo effects on
449 peak minute power during incremental arm crank ergometry. *Eur J Sport Sci*
450 2014; 14(4): 362-367.
- 451 6. Bühler ES, Dirk WLS, Schlegel KG, Winkler S. Development of a tool to
452 assess the caffeine intake among teenagers and young adults. *Ernaehrungs*
453 *Umschau* 2014; 61(4): 58–63.
- 454 7. Clark VR, Hopkins WG, Hawley JA, Burke LM. Placebo effect of
455 carbohydrate feeding during a 4-km cycling time trial. *Med Sci Sports Exerc*
456 2000; 32:1642-1647.
- 457 8. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. 2nd ed.
458 Hillsdale (NJ): Lawrence Erlbaum Associates; 1988. p. 20.
- 459 9. de la Fuente-Fernandez R, Phillips AG, Zamburlini M, Sossi V, Calne DB,
460 Ruth TJ., Stoessl AJ. Dopamine release in human ventral striatum and
461 expectation of reward. *Behav Brain Res* 2002; 136(2): 359-63.

- 462 10. Fred Hutchinson Cancer Research Center Web site [Internet]. Seattle (WA):
463 Specific Food Questionnaire: Caffeine Questionnaire. Available from:
464 <http://www.fredhutch.org>.
- 465 11. Graham TE, Spriet LL. Metabolic, catecholamine, and exercise performance
466 responses to various doses of caffeine. *J Appl Physiol* 1995; 78(3): 867-874.
- 467 12. Hopkins WG. Probabilities of clinical or practical significance. *Sportscience*
468 2002; 6, 431 Available from: <http://www.sportsci.org/jour/0201/wghprob.htm>.
- 469 13. Hopkins WG. How to interpret changes in an athletic performance test.
470 *Sportscience* 2004, 8(1), pp.1-7.
- 471 14. Jeukendrup AE, Hopkins S, Aragón-Vargas LF, Hulston C. No effect of
472 carbohydrate feeding on 16 km cycling time trial performance. *Eur J Appl*
473 *Physiol* 2008; 104(5): 831-837.
- 474 15. Lundby C, Millet GP, Calbet JA, Bärtsch P, Subudhi AW. Does ‘altitude
475 training’ increase exercise performance in elite athletes? *Brit J Sport Med* 2012;
476 14: bjsports-2012.
- 477 16. Mason RL, Gunst RF, Hess JL. Statistical design and analysis of experiments:
478 with applications to engineering and science. 2nd ed. John Wiley & Sons, Inc,
479 Hoboken, New Jersey; 2003, p. 328-31.
- 480 17. McLaughlin JE, King GA, Howley ET, Bassett DR Jr, Ainsworth BE.
481 Validation of the COSMED K4 b2 Portable Metabolic System. *Int J Sports*
482 *Med* 2001; 22: 280-284.
- 483 18. Paton CD, Hopkins WG. Variation in performance of elite cyclists from race
484 to race. *Eur J Sport Sci* 2006; 6(1): 25-31.
- 485 19. Pollo A1, Carlino E, Vase L, Benedetti F. Preventing motor training through
486 nocebo suggestions. *Eur J Appl Physiol* 2012; 112(11): 3893-903.

- 487 20. Reilly T, Brooks GA. Exercise and the circadian variation in body temperature
488 measures. *Int J Sports Med* 1986; 7(6): 358-362.
- 489 21. Spriet LL. Exercise and sport performance with low doses of caffeine. *Sports*
490 *Med* 2014; 44(2): 175-184.
- 491 22. Yang EV, Bane CM, MacCallum RC, Kiecolt-Glaser, JK, Malarkey, WB,
492 Glaser, R. Stress-related modulation of matrix metalloproteinase expression. *J*
493 *Neuroimmunol* 2002; 133 (1-2): 144-50.
- 494

495 **Authorship information**

496 Significant manuscript writer – Bryan Saunders

497 Significant manuscript reviewer/reviser – Bruno Gualano, Hamilton Roschel,

498 Guilherme Giannini Artioli

499 Concept and design – Bruno Gualano, Bryan Saunders, Hamilton Roschel, Guilherme

500 Giannini Artioli

501 Data acquisition – Bryan Saunders, Luana Farias de Oliveira, Rafael Pires da Silva,

502 Vitor de Salles Painelli, Livia Souza Gonçalves, Guilherme Yamaguchi, Thiago

503 Mutti, Erika Maciel

504 Data analysis and interpretation – Bryan Saunders, Luana Farias de Oliveira, Rafael

505 Pires da Silva, Livia Souza Gonçalves, Guilherme Yamaguchi, Thiago Mutti, Erika

506 Maciel

507 Statistical expertise – Bryan Saunders, Hamilton Roschel, Vitor de Salles Painelli

508

509 **Figures**

510 Figure 1. Effect sizes compared to CON in CAF and PLA separated into subgroups
511 based upon supplement identification pre- and post-exercise. Panel A displays CAF vs.
512 CON pre-exercise. Panel B displays CAF vs. CON post-exercise. Panel C displays
513 PLA vs. CON pre-exercise. Panel D displays PLA vs. CON post-exercise.

514

515 Figure 2. Individual percentage change from CON in CAF (Panel A) and PLA (Panel
516 B) organised according to supplement identification subgroups pre- and post-exercise.
517 The grey dotted line represents the natural variation of the test ($\pm 3.0\%$) while the
518 black dotted line represents the mean overall improvement with caffeine ($+4.1\%$). The
519 number of participants who improved above, were within, or worsened beyond the
520 natural variation of the test in each subgroup is displayed below each graph.

521

522 **Table 1.**¹
523

Characteristic	Mean (SD)	Range	
		Minimum	Maximum
Age (y)	37 (8)	18	55
Height (cm)	1.76 (0.06)	1.60	1.89
Body mass (kg)	74.3 (8.4)	58.9	93.0
Experience (y)	12 (11)	1	40
Weekly training	Duration (h)	4	25
	Distance (km)	50	500
VO _{2max}	Absolute (L·min ⁻¹)	2.8	4.8
	Relative (ml·kg·min ⁻¹)	33.6	64.5
HR _{max} (beats·min ⁻¹)	182 (11)	158	201
W _{max}	Absolute (W)	181.4	439.0
Habitual caffeine intake (mg·day ⁻¹)	192 (156)	1.77	583.0

524

¹ Table 1. Participant characteristics

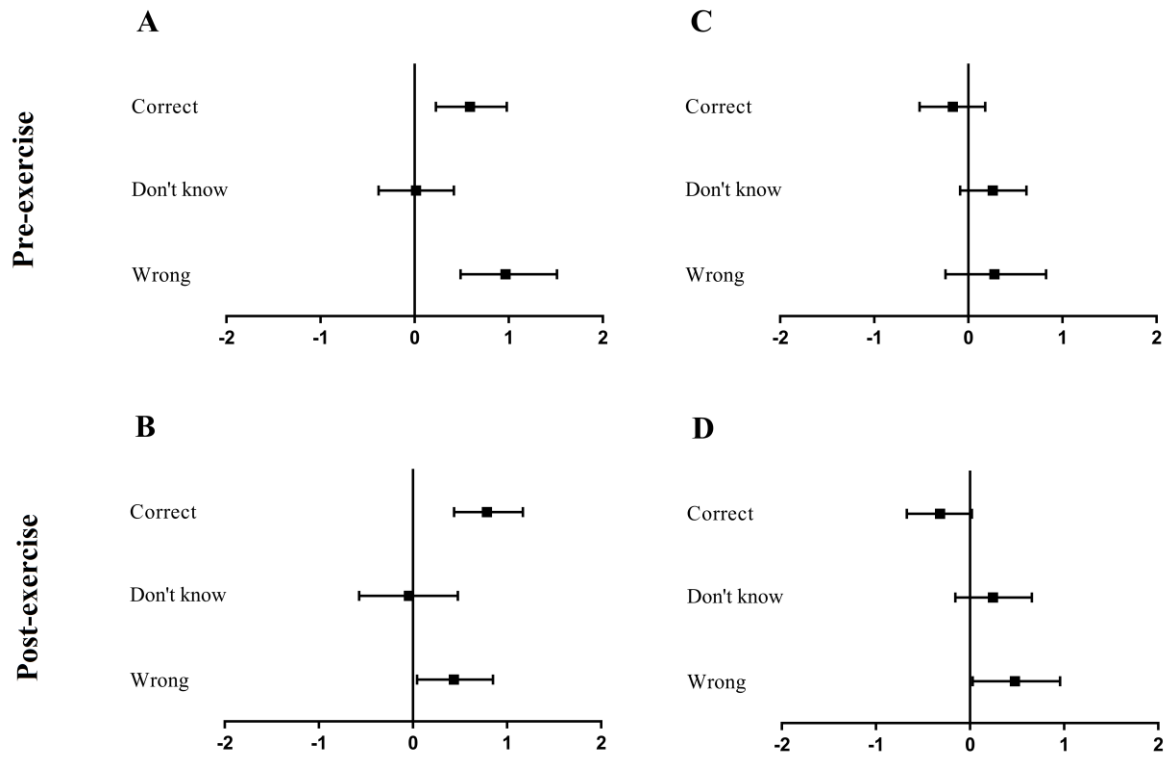
525 **Table 2.²**
 526

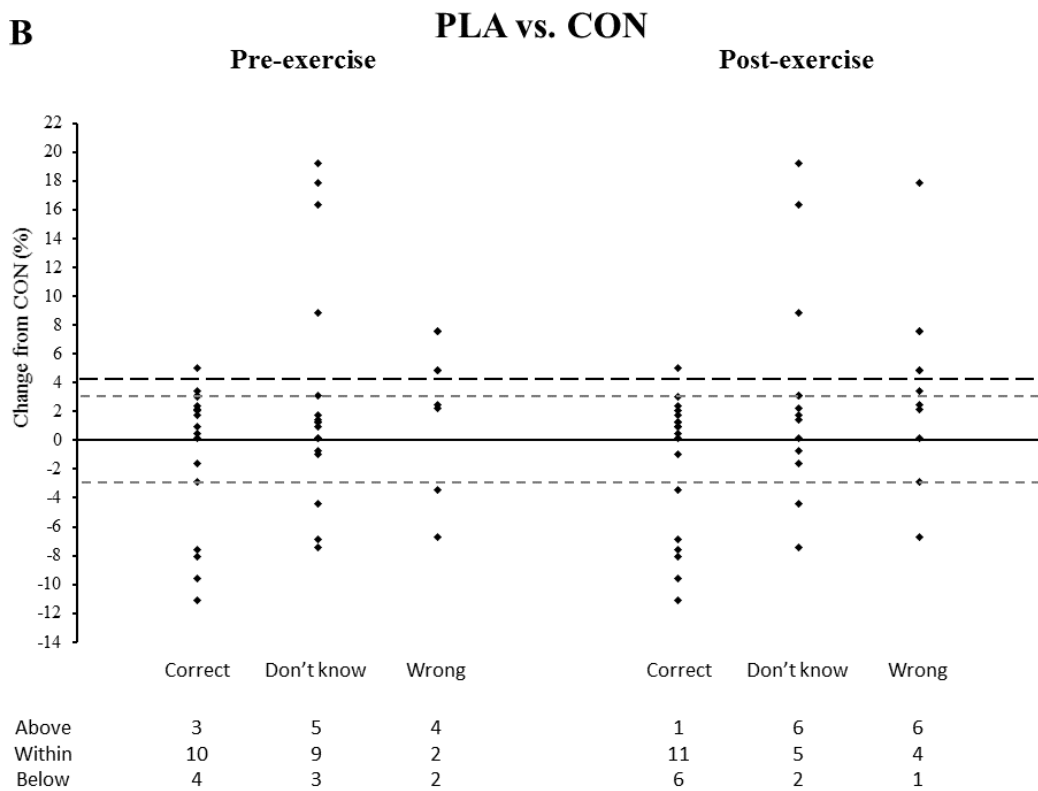
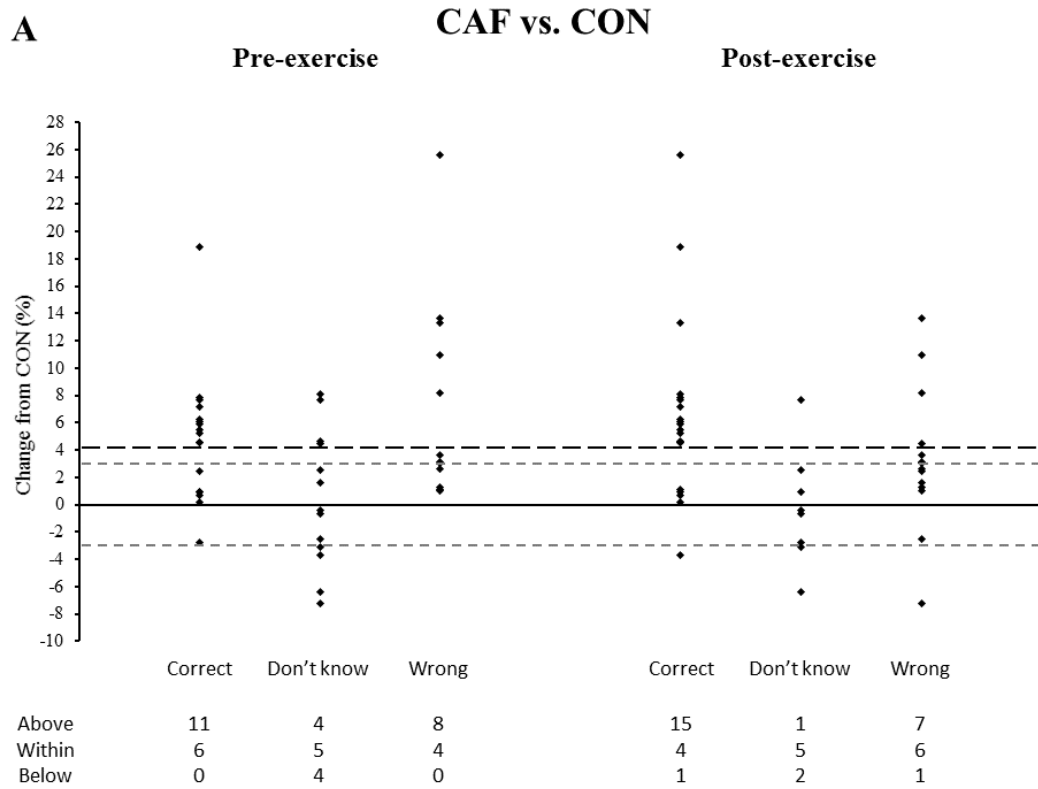
		Pre-exercise identification			Post-exercise identification		
		Correct	Don't know	Wrong	Correct	Don't know	Wrong
	N	17	13	12	20	8	14
CAF	MPO (W)	233.9 ± 41.1*	230.9 ± 32.6	238.2 ± 37.1 [^]	236.4 ± 37.2*	234.1 ± 35.8	231.1 ± 39.0 [#]
CON		223.7 ± 40.7	230.8 ± 36.0	223.2 ± 40.3	223.2 ± 39.7	236.0 ± 43.0	223.6 ± 35.6
CAF vs. CON	% difference	+4.8 ± 4.7	+0.4 ± 5.0	+7.3 ± 7.5	+6.5 ± 6.6	-0.3 ± 4.2	+3.3 ± 5.2
MBI	% chance of being beneficial/trivial/harmful	99/1/0	13/76/11	99/1/0	100/0/0	7/65/28	87/13/0
	N	17	17	8	18	13	11
PLA	MPO (W)	240.0 ± 40.8	226.5 ± 30.0	205.6 ± 38.3	230.5 ± 43.4	223.6 ± 36.9	229.0 ± 30.1 ^{\$}
CON		242.0 ± 36.9	221.4 ± 35.8	200.5 ± 34.4	233.8 ± 39.9	218.3 ± 42.7	221.3 ± 30.6
PLA vs. CON	% difference	-1.0 ± 5.0	+3.0 ± 8.0	+2.4 ± 5.1	-1.6 ± 4.9	+3.2 ± 7.6	+3.7 ± 6.3
MBI	% chance of being beneficial/trivial/harmful	3/72/25	62/37/1	61/37/2	0/60/40	62/36/2	84/16/0

² Table 2. MPO in CAF, PLA and CON, and % absolute difference from CON in CAF and PLA, when categorising individuals into their pre- and post-exercise supplement identification responses. *P ≤ 0.001 from CON. [^]P ≤ 0.01 from CON. [#]P ≤ 0.05 from CON. ^{\$}P ≤ 0.06 from CON. MPO = Mean power output; CAF = Caffeine trial; PLA = Placebo trial; CON = Control trial; MBI = Magnitude based inferences.

CAF vs. CON

PLA vs. CON





531
532
533

Figure 2.

